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# STANDARDIZATION OF THYROID PREPARATIONS*

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Although thyroid is one of the most valuable and specific drugs used in therapeutics, its dosage is largely empiric: a given preparation is administered until the desired effect is obtained, but the dosage thus determined does not hold for other preparations and sometimes, as will be shown below, not even for another preparation of the same manufacturer, although it may bear the same label. The doses mentioned in clinical reports are usually meaningless; they seldom convey useful and sometimes convey misleading information. The same is true of the labels on most of the commercial preparations.

This state of affairs, which would be considered intolerable in the case of other important drugs, results from the fact that there is no generally accepted standard for thyroid. There are, moreover, peculiar difficulties in fixing either a chemical or physiologic standard for this drug. Present knowledge of the "active principle" or of principles of thyroid does not aid very materially in the fixing of standards. It is not certainly known in what form the active agent reaches the blood from the gland or from the intestinal tract when the drug is administered by mouth. There is little doubt that the active agent is contained in the iodothyroglobulin of the gland, and the work of Hektoen, Carlson and Schulhof [1] would suggest that this is secreted, to some extent at least, by the gland. There is no evidence, however, that iodothyroglobulin when it reaches the blood is itself physiologically active; I have found that of a foreign species (sheep), when injected intravenously into mice, to be entirely inactive as tested by the delicate acetonitril method (described below). The same preparation given in the same doses by mouth was approximately as active as the entire gland when administered in equiiodin doses.[2] Although the "active principle" is contained in the thyroglobulin, probably as a part of the

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1. Hektoen, L.; Carlson, A. J., and Schulhof, Kamil: Precipitin Reaction of Thyroglobulin, Presence of Thyroglobulin in Thyroid Lymph of Goitrous Dogs, J. A. M. A. **81**:86 (July 14) 1923.

2. I am indebted to Dr. A. T. Cameron for placing the preparations of iodothyroglobulin at my disposal.

molecule, the determination of the amount of this globulin in a gland cannot be used as a method of assay, for the physiologic activity of different specimens of thyroglobulin varies greatly (according to the amount of iodin present). The amount of "thyroxin" (a definite iodin compound which may be obtained from thyroid and which seems undoubtedly to be the essentially active group in the gland) cannot be used as a basis for standardization for, at least with the present methods, there does not seem to be a constant relation between the amount of thyroxin that can be obtained from a gland and the physiologic activity of the latter.

The proposal has been made, but apparently never put into practice, that the gland be standardized by physiologic methods, and a number of these have been suggested. The best of these methods would seem to be the determination of the effects on the basal metabolism in cases of myxedema,[3] although probably similar experiments on thyroidectomized animals would be satisfactory.

The U. S. P. IX, on the suggestion of Hunt and Seidell, adopted a standard based on the iodin content of the gland, but this standard has been adopted by only a few manufacturers, probably because most clinicians are satisfied to use unstandardized preparations; unsatisfactory results with commercial preparations are usually attributed, without any demonstrated basis in fact, to the age of the preparation, improper methods of manufacture or to some idiosyncrasy on the part of the patient. Moreover, doubts have been expressed recently as to whether the iodin content of the gland is really a measure of its physiologic activity; the belief that thyroid contains physiologically "inactive iodin" in large but variable amounts has been strengthened in some quarters by the fact that only a fraction of the iodin can be secured in combination as thyroxin. It is obviously impossible, however, to draw any safe conclusions as to the physiologic activity of the iodin combination in the thyroid from the products obtained by treating the gland with sodium hydroxid.

I have pointed out, in earlier papers, that there is a close parallelism between the iodin content of different preparations of thyroid and their various physiologic effects; Cameron[4] has recently summarized the literature on this subject and, with Carmichael, introduced a new method for studying the problem.

### INACTIVE IODIN

If, however, the iodin content of the thyroid is to be used as the basis for standardization, it is necessary to be certain that glands which are to be used in medicine do not contain "inactive iodin," i. e., inactive

3. Comparisons should be made with Kowitz: Ztschr. f. d. ges. exper. Med. **34**:457, 1923.

4. Cameron, A. T.: Canad. M. A. J. **14**:407 (May) 1924.

in the sense that some of the iodin is not present in the combination producing the typical effects of thyroid.

*Experiments.*—In investigating this subject experimentally, I have continued to make use of the "acetonitril reaction" (the increased resistance to acetonitril produced in white mice by the administration of thyroid); this seems to be the simplest and most delicate of the tests on animals proposed. The results are, moreover, in entire harmony with the results of other methods and also with clinical experience.

I [5] have recently discussed certain criticisms that have been made of this method, and emphasized that the test is "specific" only in the sense that an increased metabolic rate is a specific test for hyperthyroidism; in either case, certain other factors must be eliminated. For just as muscular activity, the administration of epinephrin and of certain foods increase the metabolism, so there are factors other than thyroid which will alter the resistance of mice to acetonitril. However, in actual practice there is no more difficulty in determining whether thyroid increases in a specific way the resistance of mice to the nitril than there is in determining whether it is the thyroid which increases the basal metabolism when administered to a patient with myxedema: in the one case, a decigram or two of thyroid is given daily to a patient receiving several hundred grams of food, and in the other a few tenths of a milligram of thyroid are given to a mouse receiving several thousand milligrams of food and the effects of the nitril determined on a group of mice that have received only the food, and on other groups of mice receiving both the food and the minute amounts of thyroid.

In my earliest papers on this subject I did show, however, that certain other glands (testis, ovary, prostate and mammary) when fed to mice increased the resistance to the nitril, but the doses necessary were hundreds or even thousands of times larger than those of the thyroid; there was never the slightest difficulty of distinguishing between thyroid and these other glands. Gellhorn [6] has recently found that "optones" obtained from some of these glands and also from the thyroid cause a slight increase in the resistance of mice to acetonitril, and concluded that the nitril test is not specific for thyroid. Gellhorn, however, injected from 200 to 300 mg. of the optones subcutaneously, and the degree of increased resistance he obtained was far less than that which I constantly obtain when a few tenths of a milligram of the entire thyroid is fed; the effect was also different from that of thyroid, for it continued for several weeks after the injections of the optones were discontinued. So far as I can learn, no evidence has been published to show that these thyroid "optones" have the action most characteristic

5. Hunt, Reid: Am. J. Physiol. **63**:257 (Jan.) 1923.
6. Gellhorn: Arch. f. d. ges. Physiol. **200**:571, 1923.

of thyroid, viz., the increase in basal metabolism especially marked in cases of myxedema. (A review of Zondek's "Die Krankheiten der endokrinen Drüsen" states that this author found the thyroid optones to have no action on basal metabolism.[7]) On the other hand, I found products of the pancreatic and peptic digestion of thyroglobulin to cause a marked increase in resistance to the nitril when fed in doses of from 0.3 to 0.5 mg. a day for five days.

I have applied the acetonitril test to about thirty thyroids of adult animals, and have not found a single case in which the degree of resistance produced was not closely proportional to the iodin content, although the latter varied from 0.01 to 0.531 per cent. In some cases, the parallelism was not as close as in others, but I am not certain that the variations were not within the range of the error in the method.

The value of a method of basing dosage on iodin content is best shown, however, by comparing the results of experiments in which this

TABLE 1.—*Results in Experiment 1* *

| Preparation | Iodin, per Cent. | Weight of Substance Fed per Day (for 5 Days), Mg. | Weight of Iodin in Sample per Day, Mg. | Minimal Fatal Dose of Acetonitril, Mg. per Gm. of Mouse |
|---|---|---|---|---|
| Controls | 0 | 0 | 0 | 0.45 |
| Thyroglobulin | 0.444 | 0.451 | 0.002 | 3.50 |
| Thyroxin | 65 | 0.00462 | 0.003 | 2.80 |
| Thyroid 1 | 0.531 | 0.376 | 0.002 | 3.70 |
| Thyroid 12 | 0.108 | 1.851 | 0.002 | 3.80 |
| Thyroid 12 | 0.108 | 0.376 | 0.00041 | 0.42 |

* In this experiment are included results obtained with a sample of thyroglobulin, and also with thyroxin.

method was used with those in which dosage was based on the amount of the gland (irrespective of the iodin content); in the absence of a standard, the latter method is the one naturally employed.

A typical experiment of this character is given in Table 1; others will be given later.

Thus Thyroids 1 and 12, when fed in equal iodin amounts, had practically the same protective action, the mice being protected against between eight and nine fatal doses of the nitril. When fed in equal doses of substance, Thyroid 1 protected against eight fatal doses, but Thyroid 12 had no protective action.

So far as I can discover, the only experiments reported which suggest that thyroid (that is, thyroid itself, not the products of its hydrolysis) may contain inactive iodin are some that I reported in connection with certain fetal and pathologic glands, a report on two fetal glands

7. Comparison should be made with Loewy and Zondek: Ztschr. f. klin. Med. **95**:282, 1922.

by Miura,[8] and those of Marine and Rogoff [9] on thyroids recently iodized in vivo. The latter authors, experimenting on dogs, state that only a small fraction of the iodin taken up by the thyroid after the injection of potassium iodid had been transformed into active iodin within thirty hours; the tadpole method of assay was used, a method that has been shown not to give trustworthy data as to the activity of thyroid preparations in mammals.[10] I had found that all the iodin that had been taken up by the thyroid of dogs after two doses of iodoform (given by mouth at an interval of twenty-four hours) was in the active form twenty-four hours after the second dose.

I have recently injected sodium iodid intravenously into rats and removed the thyroids at different periods and fed them to mice, and then tested the resistance of the latter to acetonitril. The thyroids removed from eight to sixteen hours after the injection of the iodid had no greater activity than the controls; those removed twenty-four hours after the injection had a marked physiologic action, which was almost, if not quite, as great as that of those removed forty-eight, seventy-two, ninety-six and 144 hours after the injection. The result was the same whether 0.3 mg. or 15 mg. of sodium iodid per kilogram had been injected, i. e., as I had shown several years ago,[11] the amount of iodin rendered "active" did not depend so much on the amount of iodin administered as on the condition of the gland (which could be readily altered by the character of the diet).

The greatest discrepancies that I have found between iodin content and physiologic activity have been in the case of fetal calf thyroids (given me by Dr. Frederic Fenger of the Research Laboratory in Organotherapeutics of Armour and Company). Some of these specimens were marked "normal," others "large," but there was no constant relation between the size of the glands and the amount of iodin and their physiologic activity. Thus a "large" fetal thyroid containing 0.01 per cent. iodid was almost as active when fed in equiiodin amounts as adult thyroids containing 0.335 and 0.536 per cent. iodin, whereas a similar gland containing 0.014 per cent. iodin had very slight activity and another with 0.05 per cent. iodin was about half as active as a normal adult gland. Of the "normal" fetal glands, one with 0.21 per cent., another with 0.26 per cent. and a third with 0.4 per cent. iodin had almost the normal activity, whereas one with 0.33 per cent. iodin had a very low degree of activity.

8. Miura, M.: J. Lab. & Clin. Med. **7**:349 (March) 1922.
9. Marine, M., and Rogoff, J. M.: J. Pharm. & Exper. Therap. **9**:1 (Oct.) 1916.
10. Swingle, W. W.; Helff, O. M., and Zwemer, R. L.: Am. J. Physiol. **70**: 208 (Sept.) 1924.
11. Hunt, Reid: Experiments on the Relation of the Thyroid to Diet, J. A. M. A. **57**:1032 (Sept. 23) 1911.

Thus, while there are thyroid glands with more or less "inactive" iodin, it is highly improbable that they would be found among those used for medicinal purposes.

*Clinical Results.*—Of course, the final solution of the question whether the standardization of thyroid preparations can be based on their iodin content must come from clinics in which careful observations on basal metabolism are made; evidence from this source is at present very scant, but what there is points very definitely to the conclusion that the iodin standard is valid, or at least that the clinical effects are far more nearly parallel to the iodin contained in the gland than to the absolute amounts of the drug administered.

Magnus-Levy [12] compared the clinical results in different forms of hypothyroidism following the administration of thyroid tablets, iodothyrin and a preparation of thyroglobulin containing 1.66 per cent. iodin; he administered the preparations, which contained very different percentages of iodin, in at least approximately equiiodin doses, and reported that, clinically, the effects of the three were the same. Magnus-Levy also reported that iodothyrin and thyroid (when given in at least approximately equiiodin doses) had the same effects on gaseous metabolism. (Other clinicians maintained that iodothyrin was less active than thyroid or thyroglobulin.)

I have recently analyzed and tested physiologically some thyroid preparations used by Dr. C. C. Sturgis in the treatment of cases of myxedema. Before treatment, the first patient had a basal metabolism of —38; for three years, his metabolism had been maintained at a level slightly below normal by the administration of thyroid. At the time of these observations, he was receiving daily 0.39 gm. of thyroid, which was found by analysis to contain 0.155 per cent. iodin; the patient was, therefore, receiving 0.603 mg. of iodin in the daily dose of thyroid, and his metabolism was —4. For a period of ten days, the patient took an average daily dose of 0.1947 gm. of a preparation of thyroid containing 0.335 per cent. of iodin; the iodin, in this dose, was 0.653 mg. The metabolism at the end of ten days was practically unchanged, being —1. Thus, the two preparations had practically the same effect on the metabolism when given in approximately equiiodin doses; the absolute amount of thyroid given, however, was twice as great in one instance as in the other. I found, in experiments on mice, that it required approximately twice as much of the iodin-poor as of the iodin-rich thyroid to cause equal degrees of resistance to acetonitril.

Three months later, the patient, whose metabolism was being maintained at about —6 by an average daily dose of 0.325 gm. of thyroid containing 0.155 per cent. of iodin, for twenty days took an average

12. Magnus-Levy: Ztschr. f. klin. Med. **52**:201, 1904.

daily dose of 0.117 gm. of thyroid with an iodin content of 0.531 per cent. At the end of twenty days, the metabolism had risen to +8, although he had taken little more than a third as much thyroid as in the preceding twenty days: he had, however, received a total of 12.5 mg. of iodin in the iodin-rich thyroid and only 10.08 mg. of iodin in the iodin-poor thyroid. Animal experiments showed these preparations to have approximately equal activity when fed in equiiodin doses, but the one to be almost three times as active as the other when administered in equal doses of the drug itself. Had the iodin-rich preparation been given to the patient in the same doses as the iodin-poor preparation, i. e., if he had received 0.325 gm. of the former, there can be little doubt that the results would have been very unpleasant, for even 0.117 gm. had had a greater effect on the metabolism than had 0.325 gm. of the iodin-poor preparation. Some of the most widely used thyroid preparations contain even higher percentages of iodin than the foregoing; this shows the danger of basing thyroid dosage simply on the amount of drug.

In the foregoing case, an iodin-rich preparation of thyroid had a greater effect on the metabolism than did an iodin-poor preparation, although the dose of the former was little more than a third as great. In the following case, an iodin-poor preparation had a slightly greater effect than one with a higher iodin content when the former was taken in doses containing somewhat more iodin than the latter. The patient, whose metabolism before treatment was —33, had been receiving thyroid for more than three years. At the time of the present observations, the metabolism was —18, and the patient was taking 0.13 gm. of thyroid daily; the thyroid had 0.155 per cent. iodin, and the patient was therefore receiving 0.202 mg. of iodin daily. He received for ten days a thyroid preparation containing 0.108 per cent. iodin in daily doses of 0.25 gm.; he therefore received 0.27 mg. of iodin daily, and the metabolism rose to —11.

Some months later, this patient took a preparation of iodothyroglobulin containing 0.444 per cent. of iodin for twenty days; the average daily dose was 0.0704 gm. containing 0.312 mg. of iodin, and this replaced an average daily dose of thyroid of 0.195 gm., which was supposed to contain 0.155 per cent. iodin and therefore 0.307 mg. of iodin. In the course of twenty days, the metabolism rose from —16 to —1. In this case, the thyroglobulin appeared to be distinctly more active, in proportion to its iodin content, than the thyroid for it seems improbable that such a small difference in the amount of iodin given could account for the difference in the effect on metabolism, if the iodin was present in the same form in both preparations. I am inclined, however, to think it equally probable that the thyroid tablets varied somewhat in iodin content, as I have found to be the case with other

thyroid tablets, and it may be that a small difference in the strength of thyroid would, in the course of twenty days, have a cumulative action sufficient to account for the difference in the effect on metabolism. Four comparisons of the activity of a sample of these tablets (obtained by grinding up ten tablets) with the thyroglobulin gave practically identical results by the acetonitril test when given to mice in equiiodin amounts; for example, in one of these tests, the minimal fatal dose of the nitril after feeding equiiodin amounts of the tablets and the thyroglobulin was 3.4 mg. per gram of mouse, whereas the controls died from 0.47 mg. per gram. The amount of thyroid substance fed daily was 1.251 mg., whereas only 0.451 mg. of the thyroglobulin was fed. In another test, however, in which tablets that had not been analyzed were compared with thyroglobulin, the latter was distinctly more active when fed to mice in amounts that were supposed to contain equal amounts of iodin; in this case, the minimal fatal dose of the nitril for the mice receiving the thyroglobulin was 2 mg. per gram, that for those receiving the tablets was 1.6 mg., whereas the control died from 0.5 mg.

There is not, however, sufficient evidence to maintain that there is an absolute parallelism between the physiologic activity of thyroid and the iodin content; that is, there is not conclusive evidence that all the iodin is in the same form of combination in the normal gland (although there is no evidence for the view that it is not). The important thing is that, with our present knowledge, a far better approximation to correct dosage can be made when this is based on iodin content than when it is based on amounts of gland administered. For example, in the foregoing case, a daily dose of 0.0704 gm. of thyroglobulin was more effective in increasing the metabolism than was a daily dose of 0.195 gm. of the preparation which the patient had been taking, or one part of the former was more than equal to 2.77 parts of the latter. If the iodin-poor preparation had been given in the same dose (i. e., 0.0704 gm.) as the iodin-rich preparation, there can be no doubt that the patient would soon have had a relapse. As a matter of fact, the patient had at one time been placed on an average daily dose of 0.087 gm. of this preparation and, within a month, his metabolism fell from —4 to —26; that is, 0.087 gm. of the iodin-poor preparation was entirely insufficient, whereas 0.0704 gm. of the iodin-rich preparation caused a greater effect than was desired. On the other hand, if the iodin-rich preparation had been given in the doses found satisfactory for the iodin-poor preparation, the results would probably have been very unpleasant. As stated above, a daily dose of 0.0704 gm. of the iodin-rich preparation had a greater (and undesired) effect on the metabolism than did 0.195 gm. of the iodin-poor preparation.

It had been found that 0.195 gm. of the iodin-poor preparation was the dose best suited to this patient; if 0.195 gm. of the iodin-rich preparation (and there are on the market preparations even more active than this) had been substituted for this, the result would have been that the patient would have received the equivalent of 0.54 gm. of the weaker preparation, for, according to the foregoing clinical and also experimental results, 1 part of the stronger preparation was rather more active than 2.77 parts of the weaker preparation. As a matter of fact, less than one half (0.26 gm. daily) of the latter had at one time been given to this patient, with the result that the metabolism rapidly rose from 32 per cent. below normal to 4 per cent. above normal and with a loss of weight of 2.7 kilograms, and the dose was therefore reduced to 0.195 gm.

I also compared the effects of two of the most widely used thyroid preparations on the resistance of mice to acetonitril, and found that approximately equal degrees of resistance were obtained when one was fed in doses from five to six times larger than the other. Analysis showed the "stronger" of these preparations to have 5.52 times as much iodin as the weaker preparation. Dr. Pelkan of the Children's Hospital found that in the treatment of a case of cretinism (a child 5 months old), these preparations had practically the same therapeutic effect when fed in equiiodin doses; the amount of thyroid administered daily in the one case (B) was 9.6 mg., whereas in the other (A) it was 53 mg. (these statements as to the amount of thyroid in the tablets are based on the assumption that the statements on the labels were correct). These doses (9.6 mg. of B. and 53 mg. of A) gave about the optimum therapeutic results; there can be little doubt that, if the doses had been based on the amount of thyroid fed, that is, if 53 mg. of B or only 9.6 mg. of A had been administered, the condition of the patient would soon have altered for the worse. Such a substitution would have been equivalent to increasing fivefold the dose of B, which was giving satisfactory clinical results, or to reducing that of A to one fifth of the dose which experience had shown to be suitable.

## COMMERCIAL PREPARATIONS OF THYROID

It is highly desirable that further clinical tests be made with doses of thyroid based on iodin content. Unfortunately, however, the available commercial preparations are not suited for such tests, as is shown by an examination of current drug catalogues. Prof. W. A. Puckner, Secretary of the Council on Pharmacy and Chemistry of the American Medical Association, kindly sent me a summary of the statements concerning thyroid contained in nineteen such circulars. In only five was the assertion made that the preparations were of U.S.P. standard. Reference was made to the iodin content in the circulars of two

other firms: it was stated that, in one, the desiccated gland contained not less than 0.3 per cent. of iodin in organic combination; in the other that each product represented the stated amount of the fresh gland, containing not less than 0.05 per cent. of iodin. Other circulars simply stated that 1 grain of the preparation, or frequently "1 tablet," represents 5 grains of the fresh gland. Physicians at the present time are about as likely to think of thyroid dosage in terms of the fresh gland as they would of the dosage of morphin in the fresh juice of the poppy. Still, the statement that 1 grain of the dried gland corresponds to 5 grains of the fresh gland, or to 5 grains of the fresh gland containing not less than a certain percentage of iodin, may indicate that approximately normal glands are used. But the statement "not less

TABLE 2.—*Analysis of Tablets Obtained from Boston Hospitals or on Open Market*

| Tablet Stated to Represent | Iodin per Tablet, Mg. | Relative Amounts of Iodin in Tablets | |
|---|---|---|---|
| | | Theoretical | Found |
| ¼ grain fresh thyroid | 0.0386 | 1 | 1 |
| ½ grain fresh thyroid | 0.0442 | 2 | 1.21 |
| 1 grain fresh thyroid | 0.0992 | 4 | 2.71 |
| 2½ grains fresh thyroid | 0.253 | 10 | 6.91 |
| 5 grains fresh thyroid | 0.439 | 20 | 11.98 |

TABLE 3.—*Analysis of Tablets Obtained in Manitoba*

| Tablet Stated to Represent | Iodin Found in Tablet, Mg. | Relative Amounts of Iodin in Tablets | |
|---|---|---|---|
| | | Theoretical | Found |
| ¼ grain fresh thyroid | 0.0283 | 1 | 1 |
| ½ grain fresh thyroid | 0.0393 | 2 | 1.28 |
| 5 grains fresh thyroid | | | |
| a | 0.387 | 20 | 13.70 |
| b | 0.410 | 20 | 14.50 |
| c | 0.400 | 20 | 14.12 |

than" so much iodin is almost as objectionable from the standpoint of rational and safe therapeutics as would be the statement that a preparation of nux vomica contains "not less than" so much strychnin.

Tablets of the same firm stated to contain equal amounts of thyroid were sometimes found to contain different amounts of iodin and "half-grain" tablets did not always contain twice as much iodin as did "quarter-grain" tablets. Thus, analysis of tablets obtained from Boston hospitals, or bought on the open market, and stated to have been made in the United States, is given in Table 2.

Cameron found similarly labeled tablets obtained from a local pharmacist in Manitoba and stated to have originated in England to contain amounts of iodin as given in Table 3.

When the quarter and half grain tablets were fed to mice in doses containing equal amounts of iodin, the effect on the resistance to acetonitril was practically the same.

When, however, in another series of experiments, the dosage was based on the amount of thyroid stated to be present, that is, when it was assumed that a half grain tablet would have the same physiologic effect as two quarter grain tablets, the quarter grain tablet caused a high degree of resistance in the mice, whereas the half grain tablet had little effect, another indication that thyroid dosage should be based on iodin content rather than on the amount of thyroid. This is of more than theoretical interest: if a physician were to substitute one of these half grain tablets for two quarter grain tablets in the treatment of a case of cretinism, for example, the progress of treatment would doubtlessly be interrupted until the proper dosage of the new preparation had been determined.

Physicians in reporting results from thyroid administration usually fail to state whether by a "2 grain thyroid tablet" they refer to a tablet containing 2 grains of dried thyroid or to one containing the equivalent

TABLE 4.—*Results When Quarter and Half Grain Tablets in Doses Containing Equal Amounts of Iodin Were Fed to Mice*

| | Iodin in Thyroid Fed Daily for 7 Days | Lowest Fatal Dose of Acetonitril per Gram Mouse |
|---|---|---|
| Control | — | 0.47 |
| ¼ grain | 0.002 mg. | 3.20 |
| ½ grain | 0.002 mg. | 3.50 |

of 2 grains of the fresh gland, or about one-fifth as much; and, since the percentage of iodin in the thyroid of the tablets that I have examined, assuming that the amount of thyroid present is correctly stated, varies from 0.155 to over 0.7, there is a possibility of one "2 grain tablet" having about twenty-five times the activity of another "2 grain tablet."

Physicians also frequently write of prescribing so many "thyroid tablets" without specifying their source or the amount of thyroid contained in them. There are on the market thyroid tablets containing the equivalent of one one-hundreth grain of the fresh gland, and also tablets containing 5 grains of the dried gland (equivalent to about 25 grains of the fresh gland), or one "tablet" may contain 2,500 times as much thyroid as another "tablet"; and since some thyroid has five times as much iodin as other preparations, and the physiologic activity is at least closely parallel to the iodin content, there is the possibility of one "tablet" having the physiologic activity of 12,500 other "tablets." [13]

13. Of course, physicians using exceptionally large or exceptionally small doses of thyroid usually state this fact, but in very many cases, in fact, in most cases, clinical statements as to the dosage of thyroid are practically worthless.

A similar condition obtains in reference to the tablets on the European market: a "0.3 gm." tablet may mean 0.3 gm. of the fresh gland; "0.1 gm." may mean 0.6 or 0.2 gm. of fresh thyroid (iodin percentage usually not stated); "0.4 gm. represents an entire fresh thyroid gland of average size," and so on. There are also on the market, both in this country and abroad, a large number of nondescript "thyroid preparations." Some of the various "thyroidproteins" seem to be simply preparations of iodothyroglobulin of various degrees of purity; those that I have tested had a physiologic activity directly proportional to their iodin content. Other preparations are aqueous and other extracts of thyroid are practically free of iodin and have, for all practical purposes, none of the action which makes thyroid such a unique and valuable drug. Such preparations and also the various mixtures of thyroid and other organs have no place in modern, rational therapeutics.

This state of confusion is very unfortunate when attempts are being made to place thyroid therapy on a rational basis, i. e., to bring the dosage into quantitative relations to the metabolism or to other factors.[14]

Reference may be made, in this connection, to experiments I have performed in which the amount of thyroid which had been found to cause a well marked increased resistance to the nitril was given in a single dose instead of in divided doses spread over five or six days; mice receiving in a single dose thyroid containing 0.0125 mg. of iodin recovered, on the fourth day, from 1.3 mg. of acetonitril per gram, whereas those receiving the same amount of thyroid in divided doses in the course of five days did not acquire a tolerance to more than 1 mg. per gram; the controls died from 0.24 mg. Thus, it may be possible to base thyroid dosage on the total amount needed, as is the case in some conditions with digitalis.

In other experiments, a physiologic sodium chlorid solution of thyroglobulin (given to me by Dr. A. T. Cameron) was used. One such experiment was as follows: Single doses of 0.2 c.c. or of 0.4 c.c. were added to a small amount of the food of a mouse and no other food was given until this was eaten (which was done in the course of a day); the usual food was then continued for four or five days, when the resistance of the mice to the nitril was determined. At the same time, similar doses of the thyroglobulin were injected intravenously into mice; they were then continued on the same food as the other mice, and the resistance to the nitril was determined at the same time.

14. Comparison should be made with: Boothby, in Oxford Medicine **3**: 950, 1922; Kowitz (Footnote 3); Nobel E., and Rosenbluth, A.: Wien. klin. Wchnschr. **37**:641 (June 26) 1924.

In another experiment, with a different preparation of thyroglobulin, the mice injected with the thyroglobulin were distinctly less resistant than the controls: thus, the latter died from 1.2 mg. of nitril per gram; those injected with 0.3 and 0.4 c.c. of the globulin died from 0.8 and 0.9 mg. of the nitril, whereas it required 1.7 mg. per gram to kill the mice given these doses by mouth at a single feeding.

*Detection of Adulterated Thyroid.*—Muscle tissue treated with a solution of potassium iodid and dried and powdered is alleged to have been sold as thyroid; many proprietary "anti-fat remedies" contain thyroid, whereas others contain potassium iodid, extract of bladderwrack and other iodin compounds. Hence, it is desirable to have tests by which nonthyroid as well as thyroid-iodin may be detected. In the case of the inorganic iodids and many of the organic iodin compounds, simple chemical tests suffice. A test of this kind was included in U. S. P. VIII; it consisted in treating a cold aqueous extract of the preparation

TABLE 5.—*Results in Experiment 21*

| | Minimal Fatal Dose of Acetonitril: Mg. per Gram Mouse |
|---|---|
| Controls; normal food | 0.55 |
| Mice injected with 0.2 and 0.4 c.c. of thyroglobulin solution | 0.55 |
| Mice fed a single dose of 0.2 c.c. of thyroglobulin solution | 1.4 |
| Mice fed a single dose of 0.4 c.c. of thyroglobulin solution | 2.3 |

with sodium nitrite, acidifying with concentrated nitric acid and shaking with chloroform. A more delicate test consists in mixing the suspected material with a drop of starch paste on a white tile; a number of the so-called iodin-protein compounds caused a blue color at once, showing the presence of free iodin, and a large number of "organic" iodin compounds and a number of "anti-fat remedies" developed a blue color on the addition of a drop of concentrated nitric acid. Some of these iodin compounds could readily be detected, in this manner, when mixed with thyroid in the preparation of 1 part to 750 parts of thyroid. Other chemical tests can be employed to distinguish between many organic iodin compounds and thyroid, but it is probable that, in some cases, the detection of foreign iodin compounds in mixtures containing thyroid would offer considerable difficulty and might be impossible at present when very small amounts of material are available. By the acetonitril method, however, it is possible to determine with a considerable degree of accuracy how much of the iodin contained in a complex mixture of iodin containing compounds is present in thyroid; this can readily be done when only a few milligrams of material are available. The principles underlying this method were discussed in

detail, several years ago, by Seidell and myself.[15] Briefly, they are as follows: No iodin compound was found which, in any dose, caused an increase in the resistance of mice at all comparable to that caused by thyroid; thus, in a large number of experiments in which a few milligrams of thyroid were fed to mice, the average degree of protection against the nitril was 13.7, that is, the mice recovered from 13.7 times the dose of the nitril fatal to the controls. No nonthyroid iodin compound was found which caused, except very rarely, an increased resistance of more than 2, even when the preparation was fed in amounts containing hundreds or thousands of times as much iodin as the thyroid. We suggested that the nonthyroid iodin compounds caused an increased resistance only through their iodin being available for increasing the active principle in the mouse's own thyroid; we showed that the extent to which this occurred was determined by the condition of the mouse's own thyroid, and that this could be modified by changes in the diet.

There were, however, differences between iodin compounds in their action in increasing the resistance of mice to the nitril and also in increasing the activity of the thyroids of rats to which they had been administered. Thus, some extracts of bladderwrack had a slight protecting action when fed to mice in amounts containing only from fifteen to thirty times as much iodin as in the minimum amount of thyroid which was detectable by the test; with no dose of bladderwrack, however, was it possible to more than double the resistance of the mice. It was necessary, in these experiments, to give very many times as much iodin in the form of potassium iodid to secure any detectable effect; thus, the iodin in bladderwrack seemed to be more available as a source of iodin for the thyroid than was that of potassium iodid. (Such iodin compounds were called "thyreotropic.") Some iodin compounds seem unable to serve as a source of iodin for the thyroid; tetraiodophenolphthalein,[16] for example, when fed to mice had no effect on their resistance to the nitril nor, when injected intravenously into rats, did it increase the activity of the thyroids of these animals (as was the case when sodium iodid was injected).

When the foregoing principles were applied to mixtures of thyroid and other iodin compounds, there was no difficulty in detecting a few thousandths of a milligram of nonthyroid iodin or a few thousandths of a milligram of thyroid-iodin, although this was mixed with thousands of times as much nonthyroid iodin. In Table 6 are given typical experiments of this character.

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15. Hunt, Reid; and Seidell, A.: J. Pharm. & Exper. Therap. **2**:15, 1910.

16. This compound was studied because it has been found to be a constituent of a widely advertised "anti-fat remedy" (J. A. M. A. **82**:734 [March 1] 1924); it was thought that it might have some especial relation to the thyroid as has the iodin compound of some extracts of bladderwrack.

We may suppose that, in Series 1 of Table 6, *b* had been prepared from thyroid known not to be adulterated with any foreign iodin compound and that *c* (an unknown) had been found by analysis to contain 0.218 per cent. of iodin; when fed in daily doses containing 0.00218 mg. of iodin, it had slightly less activity than 0.0011 mg. of iodin in *b*, and we would conclude that not more than half the iodin was in thyroid combination; about a fourth the iodin in *d*, none of that in *e* (although it had nearly 4,000 times as much iodin as *b*) and all that in *f* would be considered to be in thyroid combination.

In Series 4, there would be some doubt as to whether all the iodin in *e* was present in thyroid because the protection afforded by thyroid was probably near a maximum with a smaller amount of iodin; this question could be answered by repeating the experiment, feeding only as much iodin of the unknown *e* as was contained in *b*, when the degree of protection should be practically the same.

TABLE 6.—*Results in Additional Experiments*

| Series | Drug or Mixture Fed | Mg. Iodin Fed Daily as | | Total Mg. Iodin in Drug or Mixture Fed | Fatal Dose of Aceto-nitril in Mg. per Gm. | Ratio of Increased Protection |
|---|---|---|---|---|---|---|
| | | Thyroid | Other Compound | | | |
| 1 a | Controls | 0 | 0 | 0 | 0.18 | 1.0 |
| b | Thyroid alone | 0.0011 | 0 | 0.0011 | 0.95 | 5.3 |
| c | Thyroid plus potassium iodid | 0.0011 | 0.00108 | 0.00218 | 0.77 | 4.3 |
| d | Thyroid plus potassium iodid | 0.0011 | 0.0029 | 0.004 | 0.98 | 5.4 |
| e | Potassium iodid alone | 0 | 3.82 | 3.82 | 0.20 | 1.1 |
| f | Thyroid alone | 0.002 | 0.0 | 0.002 | 1.8 | 10.0 |
| 4 a | Controls | 0 | 0 | 0 | 0.54 | 1.0 |
| b | Thyroid alone | 0.00053 | 0 | 0.00053 | 2.0 | 3.7 |
| c | Thyroid plus potassium iodid | 0.00053 | 0.1986 | 0.19913 | 2.2 | 4.1 |
| d | Potassium iodid alone | 0 | 0.1986 | 0.1986 | 0.56 | 1.+ |
| e | Thyroid alone | 0.0015 | 0 | 0.0015 | 4.0 | 7.4 |

Many similar experiments were performed in which iodin compounds other than potassium iodid were used; the results were the same. Dr. Seidell also prepared a number of mixtures similar to the above and, without knowing their composition, I was able to determine the approximate amount of thyroid-iodin contained in them; many experiments involving the use of a large number of mice were, however, necessary.

It is of interest to note in connection with the recent use of iodin,[17] in the treatment of exophthalmic goiter that in these experiments we did not find nonthyroid iodin to have a distinct or constant antagonistic action to the thyroid; usually, the foreign iodin compound, when fed in relatively large doses, slightly increased the protection afforded by the thyroid.

17. Loewy, A., and Zondek, H. (Deutsch. med. Wchnschr. **47**:1387 [March 31] 1921) used potassium iodid; Plummer and others in this country use a preparation containing free iodin.

These results are somewhat analogous to those recently reported by Starr, Segall and Means,[18] who found that the administration of iodin did not reduce the thyrotoxicosis from the ingestion of thyroid, and those of Sturgis (quoted by these writers) who found, in experiments on rabbits, that previous iodinization had no influence on the toxic effects of thyroxin.

I also found that the activity of rats' thyroids (determined by removing and feeding them to mice) was not altered by the intravenous injection, six hours before the rats were killed, of large doses of sodium iodid; this was also true when the activity of the rat thyroids had been increased about twofold by the intravenous injection, forty-eight hours previously, of small doses of sodium iodid. On the other hand, I did find, in some experiments planned to test the suggestion made by Oswald that iodin increases the secretion of the thyroid gland, as well as makes the secretion more active, that the intravenous injection of sodium iodid into mice lowered their resistance to acetonitril for a day or two, results which suggest that possibly the iodids have an inhibitory effect on thyroid secretion. The latter suggestion has been made, and rejected, by Hildebrandt,[19] who found that "small" doses of potassium iodid lowered the metabolism not only of normal but also of thyroidectomized rats. The doses used by Hildebrandt for rats were, in proportion to body weight, from six to 300 times as large as those which I used for mice.

## SUMMARY

The physiologic activity of thyroid preparations as determined by the acetonitril test on mice and by clinical observations was found to be closely parallel to the iodin content.

Little or no physiologically inactive iodin was found by these tests in the thyroids of adult animals.

Very small amounts of nonthyroid iodin in adulterated thyroid preparations could be detected by the acetonitril test and also by simple chemical tests.

Very few of the thyroid preparations on the market comply with the U. S. P. standard; they were found to vary greatly in physiologic and therapeutic activity.

18. Starr, Paul; Segall, H. N., and Means, J. H.: The Effect of Iodin in Exophthalamic Goiter, Arch. Int. Med. **34**:355 (Sept.) 1924.
19. Hildebrandt: Arch. f. exper. Path. u. Pharmakol. **96**:292, 1923.

# NAUSEA AND RELATED SENSATIONS ELICITED BY DUODENAL STIMULATION

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The purpose of this paper is to present the results of a clinical and laboratory study of sensation arising from stimulation of the duodenum. The chief of these sensations is nausea. Since nausea is a sensation, its presence must be identified with some objective or motor phenomenon before it can be studied by animal experimentation. Up to the present, no adequate studies dealing with the mechanism of nausea have been noted in the literature. I therefore believe that the present experiments open up a field for investigation, and hope that the data presented may stimulate further work on this important subject.

## METHODS

The present study was made on a series of sixty ambulatory patients, the majority of whom complained of gastro-intestinal disturbances. In the number were many women, and prominent among their complaints were symptoms suggestive of circulatory instability. The Rehfuss tube was used for duodenal drainage. The stomach was usually washed with warm water, and then the bulb was allowed to pass into the duodenum. Four test solutions were injected serially: 30 per cent. magnesium; 15 per cent. disodium phosphate; 2 per cent. hydrochloric acid, and 2 per cent. sodium bicarbonate. In considering the method of drainage employed, it is evident that we are dealing, on the one hand, with certain mechanical stimuli, such as the presence of the bulb in the duodenum and the distention of the intestine with the injected fluids, and, on the other, with chemical stimuli. It soon became evident that 2 per cent. hydrochloric acid and 2 per cent. sodium bicarbonate had no effects demonstrably different from the other solutions, so the use of these was discontinued.

## EXPERIMENTAL RESULTS

*The Production of Nausea.*—The data which have been collected will be used to illustrate the points under discussion, since they do not lend themselves readily to statistical presentation. Some fifteen to twenty minutes were usually consumed in swallowing the tube and washing the stomach. It was customary, at this point, to have the patient continue swallowing the tube until the duodenal mark was reached. During these swallowing movements and after they were completed, a conversation was kept up in order to prevent, as far as possible, the development of psychic inhibitory impulses to stomach and pylorus. It was no uncommon experience to have such a conversation interrupted without warning

by a transitory wave of nausea. This would be followed by a flow of bile stained material from the tube. Sometimes a change of position from the supine to the erect or from the right side to the prone precipitated such an attack. Indeed the appearance of a transitory attack of nausea came to be regularly regarded as a signal that the bulb had passed into the duodenum, and that now the test solutions might be injected.

In Case 19, the bile was flowing freely. Magnesium sulphate was injected and was followed by a wave of nausea. The reaction of the material draining from the tube became strongly acid. This experience recurred three times before a satisfactory sample of bile was obtained. As a working hypothesis, it was assumed that, with each wave of nausea, the bulb of the tube had been returned to the stomach. In Cases 225 and 255, this interpretation was checked by means of a fluoroscopic examination. A satisfactory sample of bile was obtained in each case, and then an injection of magnesium sulphate in one case, and sodium phosphate in the other, was made. Immediately, a wave of nausea resulted, and the reaction of the material draining from the tube became strongly acid. The fluoroscope now showed that the bulb was lying in the stomach. The most striking example of nausea was found in Case 6. The patient had been a sufferer from headaches, dysmenorrhea and indefinite gastro-intestinal symptoms for a long time. The gallbladder and appendix had been removed six months previously. More recently, she had complained of daily attacks of dizziness. After swallowing the bulb, the patient was quite comfortable for a period; then nausea of a low grade, accompanied by dizziness, manifested itself. Magnesium sulphate was injected, drop by drop, and a typical flow of bile resulted. On the completion of this collection and while the nausea still persisted, the tube was withdrawn to the stomach mark. Nausea ceased at once, but the dizziness persisted for a period. There can therefore be no doubt that nausea results from the use of the tube in the manner described. It is next important to determine the origin of the stimulus producing the sensation.

*Does the Sensation Arise from the Pharynx?*—It is a well established fact that stimulation of the pharynx readily leads in many subjects to vomiting. Indeed, it is difficult for the patient to swallow the bulb on the first trial because of this factor. However, when the bulb is once in the esophagus, the tendency to vomit lessens. Only occasionally is a patient found whose pharynx is so sensitive as to resent the presence of the tube, once it is in place. If these patients are asked relative to the sensation which arises from the presence of the tube, they speak of it as "gagging" and state that there is a feeling that they are going to vomit. They do not characterize it as nausea. Any observer may readily verify these statements on himself, as I have done. The injection, through the

tube, of water of distinctly different temperature from that of the body may elicit unpleasant sensations to which no immediate description is applicable, but these sensations are not nausea. In fact, it is hard to see how a constant stimulus, such as the presence of a tube in the pharynx, would be interpreted at unexpected moments as a sensation of nausea.

*Does the Sensation Arise from the Stomach?*—The stomachs of all the patients were washed as a matter of routine. At times the washing was vigorous. Water of varying temperatures was used. During this process, nausea was elicited in no instance. In fact, it is a matter of clinical experience that in the presence of persistent nausea, gastric lavage does not increase nausea but rather relieves it. I [1] have washed the stomachs of a large number of dogs and rabbits through gastrostomy openings. If the stomach of the dog was distended unduly, he would gulp once or twice and empty it through the mouth. There was no salivation or licking of the nose which would indicate the presence of nausea.

*Does the Sensation Arise from the Duodenum?*—A consideration of the evidence presented, in the cases cited and others of the same series, leaves no doubt as to the location of the bulb in the duodenum at the time nausea appeared. It was decided, however, to check this point on a dog with a gastrostomy. The bulb was introduced, under ether anesthesia, into the duodenum, and the rubber tube was brought to the abdominal surface through the gastrostomy wound. The next day, the stomach was washed thoroughly, even roughly, with a second tube introduced into the gastrostomy. Into the latter tube, also, the test solutions were injected. Finally, with the palpating finger, the duodenal tube was traced to the pylorus. From none of these manipulations did salivation or licking of the nose (evidence of nausea) result. At this point, 5 c.c. of 15 per cent. disodium phosphate was injected rather rapidly into the duodenal tube. The animal began to salivate, licked his nose, and gave a few retching movements, but he did not vomit. I think it is safe to say that, in this case, we have an experiment which duplicates the clinical ones cited above. On another occasion, an attempt was made in the same animal to introduce beyond the pylorus a Rehfuss tube and a tube carrying a rubber balloon, so that tracings of the movements of the duodenum could be registered. As soon as the animal waked from the anesthetic, an antiperistaltic wave returned both tubes to the stomach. This occurred despite the fact that the balloon and bulb were placed well beyond the pylorus, presumably close to the jejunum. This irritability of the duodenum has been noted by other workers. Thus,

1. Keeton, R. W., and Koch, F. C.: Am. J. Physiol. **37**:481, 1915. Luckhardt, A. B.; Keeton, R. W.; Koch, F. C., and La Mer, Victor: Am. J. Physiol. **50**:527 (Jan.) 1920. Keeton, R. W.; Koch, F. C., and Luckhardt, A. B.: Am. J. Physiol. **51**:454; 469 (April) 1920.

Luckhardt, Phillips and Carlson,[2] in their studies on the pylorus, observed that reflex emesis is certainly more readily elicited from mechanical irritation of the duodenal mucous membrane near the pylorus than from simple irritation of the gastric mucosa.

The ease with which nausea is elicited appears to be a rough index of duodenal irritability. Subjects having the greatest irritability show nausea when the bulb of the tube is either present in the duodenum or moved slightly. Others, having less irritability, respond with nausea when the fluid is injected sufficiently rapidly to cause a distention of the intestine. Still others have nausea only after a definite latent period has elapsed subsequent to the injection of magnesium sulphate or disodium phosphate. Of these two, the disodium phosphate is more potent in producing the symptoms, but it has less effect in establishing a flow of bile. The presumption therefore is strong that, in searching for the origin of nausea, attention will be fixed primarily on the movements of the duodenum rather than on a mechanism which is effective in causing the discharge of bile. Thirty-four of the cases examined showed no nausea or other symptoms. Even in the same patient, there were variations from day to day in the ease with which symptoms were elicited.

*Other Symptoms Elicited by Duodenal Stimulation.*—Dizziness was the next most definitely described symptom that resulted from the use of the tube. Five of the twenty-six cases that showed symptoms complained of it. One patient (Case 6) described the dizziness as coming in waves; another (Case 19) spoke of it as "light headedness." In two of the cases (Cases 124 and 204), nausea also was present with the dizziness. In the others, dizziness appeared without the nausea. In Case 6, the dizziness persisted until the tube was completely removed from the mouth while the nausea disappeared when the bulb was drawn back into the stomach. The same patient had intermittent attacks of dizziness during the remainder of the day. In the other cases, the dizziness was quite transitory.

*Pressure Sensations Referable to the Head.*—There was a third group of sensations which have been variously described by the patients, but which, for convenience, may be grouped under the term of "pressure sensations." This term, pressure, is involved in all the descriptions given by the patients, and in using it there was no intention of conveying the idea that there was an actual increased intracranial pressure, although such may exist. The patient was asked to describe, as accurately as possible, the sensations produced. His phraseology has been included in the accompanying table. Since the opportunity of studying these cases was often limited, the diagnoses are provisional rather than final.

2. Luckhardt, A. B.; Phillips, H. T., and Carlson, A. J.: Am. J. Physiol. **50**:57 (Oct.) 1919.

*Headache.*—In five cases (Cases 124, 156, 167, 214 and 204), the head discomfort became so sharp that the patients characterized it as true headache. Three of these patients were sufferers from migraine. In Case 167, the headache which followed the stimulation was in the same location (left eye and left occipital region) in which the migraine headaches were customarily located. In the other two (Cases 156 and 214), the location and duration of headache were different. The fourth patient (Case 204), who had been previously under treatment for duodenal ulcer, found that the duodenal drainage on one occasion gave her relief from a persistent headache. The fifth patient (Case 124) found

*Pressure Sensations Referable to Head*

| Patient | Sensation | Manipulation | Diagnosis of Case |
|---|---|---|---|
| 19 | Bigness of head | During injection of magnesium sulphate | Undetermined (cholecystitis?); postoperative adhesion |
| 38 | Fulness of head | Aspiration of duodenal contents with syringe | Hyperthyroidism; cholecystitis? |
| 96 | Pressure over top of head; tightening of band about forehead | After injection of magnesium sulphate during the resulting flow of bile | Migraine |
| 97 | Fulness over forehead and eyebrows | Distention of duodenum following gravity injection of magnesium sulphate; relieved by discontinuing injection | Hypertension; cholelithiasis proved by operation |
| 156 | Tightness between the eyes | After magnesium sulphate and disodium phosphate injections | Infected teeth; infected tonsils; menopause |
| 160 | Heavy feeling in head and over eyebrows | After magnesium sulphate injection | Undetermined; chronic appendix suspected |
| 163 | Fulness between eyes, extending to temples | Present during injection of magnesium sulphate; relieved on discontinuing injection | Colitis; etiology undetermined |
| 173 | Fulness of head | Following injection of magnesium sulphate and sodium phosphate | Cholecystitis (not operated on) |
| 255 | Heaviness about the eyes | Ten minutes after magnesium sulphate injection and during flow of bile | Chronic appendicitis; menopause; migraine |

that a slight headache, which was present at the onset of duodenal manipulation, became much worse before the drainage was terminated. In studying the rôle of the intestines in hunger with a balloon in the duodenum and one in the stomach, Ivy[3] recently observed that when the hunger period begins nausea and headache occur, and that vigorous hunger contractions do not occur because of inhibition. The distention of the duodenum by the balloon might, in this case, be regarded as the effective stimulus in causing the nausea and headache.

*Neck Sensations.*—In six of the cases (Cases 41, 96, 167, 175, 222 and 248), there were sensations referable to the neck. These were

3. Ivy, A. C.: Personal communication to the author. Data to be published soon.

described as "tightening of neck muscles, drawing of the neck, stiffness of the neck, pressure over the neck and aching of the neck." In more than one instance, it was stated that the sensation aroused a desire to massage and rub the neck.

*Syncope.*—In Case 115, a rather remarkably organized syndrome resulted from the injection of the magnesium sulphate. Shortly after the injection, the patient developed pain in the back of the head which radiated anteriorly across the eyes. This was followed by a sense of weakness, faintness and three attacks of syncope. On recovering, she stated that there was a "wooden feeling" in both legs. This patient had developed a similar spontaneous attack ten days before this experimentally produced one. This spontaneous attack was afterward followed by tenderness over an incision through which the gallbladder had been drained, and by pain beneath the right shoulder blade.

These experimental data, therefore, seem to show that the definite sensations enumerated above result from the stimulation of the duodenum. It will be noted that one of these (nausea) has a definite entity. It was quite constantly present whenever the duodenum was at all irritable. The others were less constantly present, were less definitely described by the patients, were often merged into each other, and might be accounted for on a circulatory (vasomotor) basis. Later, the question of the relation of this circulatory group of sensations to nausea will be considered more fully.

## COMMENT

*Stimulation of the Duodenum.*—It is important to know what results have been reported by other observers from duodenal stimulation.

On the motor side, it was observed by Wheelon and Thomas [4] that in anesthetized animals elevations in duodenal tonicity are associated with reductions in the height of antral waves. McClure, Reynolds and Schwartz [5] studied, under the fluoroscope, the behavior of the stomach in five men who had previously swallowed a duodenal tube. When the duodenal bulb was moved, the gastric peristalsis in two cases became shallow; in another, ceased for several minutes, and in a fourth changed into a pylorus spasm. Brunemeier and Carlson [6] have shown that stimulation of the duodenum inhibits the tonus and contractions of the empty stomach. These observations demonstrate the existence of a state that is necessary for antiperistalsis. The presence of antiperistalsis following duodenal stimulation has been previously reported in man by

4. Wheelon, H., and Thomas, E. J.: Am. J. Physiol. **59**:72 (Feb.) 1922.
5. McClure, C. W.; Reynolds, L., and Schwartz, C. O.: On the Behavior of the Pyloric Sphincter in Normal Man, Arch. Int. Med. **26**:410 (Oct.) 1920.
6. Brunemeier, E. H., and Carlson, A. J.: Am. J. Physiol. **36**:191, 1915.

Ivy [7] and by Lehman and Gibson,[8] and in the dog by Ivy and McIlvain.[9] Two other motor phenomena also have been described: a maintained contracture or tonus of the duodenum, by Wheelon and Thomas,[10] and an increase in the tone of the pylorus, by Carlson and Litt.[11]

On the sensory side, Ivy [7] noted that the injection of an alkaline solution in man gives rise to marked nausea followed in two instances by abdominal vomiting. Lehman and Gibson [8] also report nausea following the introduction of a catheter through a jejunal fistula into the duodenum. McClure, Reynolds and Schwartz [5] noted that nausea followed the withdrawal of the Rehfuss bulb into the third part of the duodenum in one of their subjects.

If nausea is to be related to a duodenal motor phenomenon, it may then originate from any one of these three activities: antiperistalsis, increased tonus of the pylorus, or increased tonus of the duodenum. Boas,[12] from purely clinical observations, considered that nausea was associated with abnormal peristalsis. Alvarez [13] believes that nausea may be one of the symptoms elicited by an intestinal antiperistalsis. He states that the vivid description, by patients, of nausea as coming in ascending waves suggests that they may be actually feeling reverse waves in the bowel. It is a common observation among roentgenologists that a sudden cessation of the antral waves with a relaxation of the fundus almost always indicates that the patient is nauseated. As previously emphasized, this relaxation is a necessary condition for antiperistalsis. Hesse [14] states that, at the beginning of nausea, there exist recognizable peristaltic waves in the antrum which are followed immediately by a contraction of the pylorus "in toto" and a relaxation of the fundus. This description would easily lend itself to a duodenal origin of the stimulus. Hatcher and Weiss [15] give experimental data showing that cats nauseated with tincture of digitalis resist strychnin poisoning much better than normal animals, which would be explained by a delay in the absorption of the strychnin. They further state that, when a nauseant is absorbed, the pylorus closes and remains closed during the continuance of nausea.

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7. Ivy, A. C.: Am. J. Physiol. **46**:340 (July) 1918.
8. Lehman, E. P., and Gibson, H. V.: Jejunal Fistula, J. A. M. A. **82**:1918 (June 14) 1924.
9. Ivy, A. C., and McIlvain, G. B.: Am. J. Physiol. **67**:124 (Dec.) 1923.
10. Wheelon, H., and Thomas, E. J.: J. Lab. & Clin. Med. **7**:375 (April) 1922.
11. Carlson, A. J., and Litt, S.: Visceral Nervous System; Reflex Control of Pylorus, Arch. Int. Med. **33**:281 (March) 1924.
12. Boas: Berl. klin. Wchnschr. **46**:1101, 1909.
13. Alvarez, W. C.: Reverse Peristalsis, J. A. M. A. **69**:2018 (Dec. 15) 1917.
14. Hesse, O.: Arch. f. d. ges. Physiol. **152**:1, 1913.
15. Hatcher, R. A., and Weiss, Soma: J. Pharmacol. & Exper. Therap. **22**:139 (Oct.) 1923; Seat of Emetic Action of Digitalis Bodies, Arch. Int. Med. **29**:690 (May) 1922.

For this statement they furnish no evidence, unless it is drawn from the work of Hesse.[14] Patients suffering from dizziness always described the sensation as of a wavelike character. Not infrequently but not so constantly, the same description is applied to nausea. It is therefore obviously impossible in the present state of knowledge to connect nausea with any particular motor phenomenon of the duodenum. This must be reserved for more accurate physiologic studies. A pathologic duodenal antiperistalsis would, however, explain the production of this symptom very satisfactorily from a clinical point of view.

*Is All Nausea of Duodenal Origin?*—Nausea may originate from widely different afferent fields—the bladder when distended, the pregnant uterus, the pelvis and the ureters of the kidney, the colon, the appendix, the heart, the brain under pressure, and any peripheral nerve when traumatized. Alvarez,[16] who regards nausea as a symptom of antiperistalsis, believes that the aboral passage of food is dependent on a metabolic gradient in the intestines. The point of high metabolism is in the jejunum, and of low metabolism in the colon. If these relations are reversed by nervous or humeral agents, there is a reversal in peristalsis and a regurgitation of chyme occurs. If a pregnant uterus can cause a contraction of the pylorus and emesis, it can as readily cause motor phenomena in the duodenum, which could be recognized as nausea. It is, of course, established that eviscerated animals [17] can be made to vomit. They also may show some of the other reflexes associated with nausea (salivation), but this does not mean that the animals have the sensation of nausea. In other words, there is nothing in the literature that makes the foregoing conception untenable. It simply must be investigated experimentally.

## THE VOMITING ACT

For an understanding of nausea and these related sensations, one must review the mechanism of vomiting. Clinically, the vomiting act is divisible into two phases, a prodromal and an emesis phase. This prodromal period manifests itself by the presence of many sensations which are difficult to describe. Chief among these is nausea. The patient may recognize waves of dizziness, or he may complain of fulness and pressure in the head. If he has a sick headache, the head pain will be present, and in some cases this, too, is relieved with the onset of emesis. It is difficult to secure sufficient cooperation from a patient to obtain an adequate description of his symptoms. He usually summarizes the

16. Alvarez, W. C.: Am. J. Physiol. **37**:267, 1915; The Motor Functions of the Intestine from a New Point of View, J. A. M. A. **65**:388 (July 31) 1915. Alvarez, W. C. and Starkweather, E.: Am. J. Physiol. **46**:186 (June) 1918. Alvarez, W. C.: Physiological Rev. **4**:352 (July) 1924.

17. Eggleston, C., and Hatcher, R. H.: J. Pharmacol. & Exper. Therap. **3**:551, 1912.

situation by saying, "I am sick." On the motor side, it will be noted that he is extremely pale, that his hands, forehead and neck are sweating, that the arrectores pili muscles are contracted with the production of the so-called "goose flesh," and that salivation is profuse. With the onset of swallowing movements, the second stage, the stage of emesis, appears. This stage has been described as a convulsive process involving the respiratory mechanism, the muscles of the esophagus and stomach, the abdomen and the body in general. This stage of emesis is predominantly a motor phase. All the sensations present prior to its onset are at once relieved. The patient is no longer weak, sick and nauseated. He is well and remains so until the cycle swings again to the prodromal phase. There may be many combinations of these two phases, and emphasis may be placed on one or the other. The prodromal phase may exist for a long period without development of emesis, or it may be so transitory as to escape notice. Such patients are then said to suffer from vomiting without nausea. Thus, in the case of rats,[15] emesis does not occur. It should be appreciated that each phase has its own pattern of reflex integration. The prodromal phase develops an evidently sensory pattern and the emesis phase, a motor one.

All the facts relative to the stage of emesis have recently been completely discussed by Hatcher.[18] With this phase of the vomiting act, the present discussion is not concerned. It is this prodromal stage, which is so easily recognized clinically, that requires a physiologic analysis. The physiologists have always started their discussions of vomiting with the movements of the stomach, as evidenced by Hatcher's recent review.[18] Clinicians, on the other hand, have been repeatedly furnishing evidence that in vomiting regurgitation of intestinal contents is an important feature of the act. Thus, Ewald[19] has reported that in ileus the stomach is filled with intestinal contents long before fecal vomiting occurs. All this evidence has been quite completely reviewed and summarized by Alvarez.[16] The motor phenomena of this phase would then consist of the establishment of intestinal antiperistalsis with a consequent regurgitation of chyme into the stomach. Logically, then, this stage might be spoken of as the "stage of regurgitation." It would be in sharp contrast to the "stage of emesis," in which the motor activity involves the gastric, esophageal, respiratory and skeletal muscles.

Some of the vomiting afferent impulses, originating in the heart and in the duodenum, have been shown by Hatcher and Weiss[15] to be mediated through the sympathetics. It would be expected that the reflex spread would be through connections most intimately associated with the sympathetic system. So the vasomotor center and vasomotor nerves

18. Hatcher, R. A.: Physiological Rev. **4**:479 (July) 1924.
19. Ewald, C. A.: Berl. klin. Wchnschr. **44**:1416, 1907.

would be involved. The resulting vascular activity doubtless explains the dizziness, weakness, pressure sensations in the neck and head, the headache and the syncope, which may be observed clinically in vomiting, and which have been produced experimentally by duodenal stimulation. The reflex spread would involve further the secretory mechanism, with resulting salivation and sweating and, later, the muscles of the hairs, with a production of "goose flesh." At this point, it should be realized that almost every writer who has discussed nausea in an experimental animal, and who has written in his protocols the words, "Nausea exists," has reached these conclusions because he has observed the animal salivating, because he has seen a look of dejection about the animal, and because he has realized that when these phenomena are present in man, *man then experiences the sensation of nausea.* This sensory prodromal stage develops all its sensations from reflexes spreading through the vegetative (sympathetic autonomic) nervous system, and for this reason they are described with difficulty. When the storm sweeps into the stomach, esophagus and skeletal musculature, then the sensations formerly present cease, and the "stage of emesis" is initiated. Hatcher and Weiss [15] have presented a teleologic view of nausea in which it was shown to be highly advantageous to a rat to delay his food in the stomach, thus rendering the absorption quite slow. With a slow absorption time, opportunity would be given for oxidation of the ingested poisons, and the rat would not secure, at any given moment, a lethal dose of toxins. The loss of vomiting then becomes an adaptation of the animal to the character of his food and the exigencies under which he secures food. While this is interesting, it in no way involves a physiologic analysis of the mechanism of nausea.

## SUMMARY

1. A study was made of the sensations arising from duodenal stimulation in a group of patients, of whom many showed symptoms of circulatory instability. Chief among these sensations was nausea. Dizziness, pressure sensations referable to the head and neck, headache and syncope also were elicited.

2. When the mechanism of the production of these sensations was considered, it was suggested that nausea resulted from a motor duodenal dysfunction, most probably from a pathologic antiperistalsis. The other sensations were regarded as of circulatory origin, and were explained on the basis of a reflex spread into the vegetative (sympathetic-autonomic) nervous system, involving primarily the vasomotor mechanism.

3. The vomiting act was considered as divisible into two stages: to the first, or prodromal stage, the name of "regurgitation" might be appropriately given, since the primary motor phenomenon of this stage consists of an intestinal antiperistalsis. Secondary motor phenomena

are to be found in salivation, sweating, circulatory and pilomotor changes. The sensations described above are regarded as those developed in the course of the convulsive spread of the vomiting act through the vegetative nervous system. The second stage, or stage of "emesis," results when the convulsion sweeps out of the vegetative system and involves the muscles of the stomach, esophagus, respiration and the skeletal muscles in general.

# THE SIGNIFICANCE OF UROBILOGEN IN THE URINE AS A TEST FOR LIVER FUNCTION

## WITH A DESCRIPTION OF A SIMPLE QUANTITATIVE METHOD FOR ITS ESTIMATION *

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Although it has long been recognized that the liver has a number of distinct functions, complete knowledge of any of these is still lacking. Especially is it difficult to determine whether any of the functions ascribed to the liver are carried on by this organ alone and not shared by other organs as well. There is far from general agreement in the interpretation of the experimental data available and from which conclusions have been drawn. In the light of this, it follows that any functional test, designed to measure liver abnormality, must be limited in its application and interpreted with great caution in terms of liver function. In any disease of the liver, it is unlikely that all functions are deranged, or at least equally involved, and, furthermore, there is no known single test which measures liver function as a whole. Liver functional tests are comparable then to renal tests, and the problem is to find simple clinical measures, each demonstrating some type of liver abnormality, and all, taken together, allowing of a general statement of liver efficiency. The present paper has to do with the clinical significance of urobilogen in the urine.

The function of the liver first recognized, and, one would assume, most easy of complete explanation, is that of bile formation and excretion. Yet this function, especially with regard to the place of bile formation, and to the relationship between blood destruction and bile pigment formation is far from being definitely established. The commonly accepted belief is that bile pigment is a product of destroyed blood cells, that the transformation occurs in the Kupffer cells of the liver, and that the hepatic cells serve the purpose of excretory cells. This belief is based largely on indirect evidence, chiefly on the chemical relationship between bile and blood pigments, and on the increase in bile pigment following abnormal destruction of blood cells. Strong opposition to the view that bile is formed only from blood cells is expressed by Whipple and Hooper,[1] who state that the feeding of blood does not increase the bile output, whereas carbohydrate feeding causes a marked increase.

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1. Whipple, G. H.: Pigment Metabolism and Regeneration of Hemoglobin in the Body, Arch. Int. Med. **29**:711 (June) 1922. Hooper, C. W., and Whipple, G. H.: Am. J. Physiol. **40**:349 (April) 1916; J. Exper. Med. **23**:137 (Jan.) 1916.

They conclude that their experiments overthrow the belief that bile pigment is formed only as a result of blood destruction. Rous and McMaster [2] criticise this conclusion, and believe that it is unwarranted because of faults in the experimental methods employed.

The belief that the liver is the sole seat of bile pigment formation is based on the classic work of Minkowski and Naunyn.[3] They found that the jaundice occurring in geese, following hemolysis from arsenuretted hydrogen did not occur in hepatectomized geese. A later worker, McNee,[4] has repeated these experiments. He found that bile could be detected in the urine of the hepatectomized geese, but only in very small amounts. In general, he confirms Minkowski and Naunyn, and goes a step further in assuming that it is the phagocytic Kupffer cells in which the transformation of hemoglobin to bile pigments takes place. He admits the possibility of the Kupffer cells in other parts of the body taking part in this transformation, but believes this can occur only to an insignificant degree.

A recent contribution to the subject is by Jones and Jones.[5] In a case of paroxysmal hemoglobinuria, they obtained a positive Gmelin test in the blood serum taken from a ligated extremity, some time after exposure to the cold. They consider this positive evidence of the formation of bile pigment outside the liver. This conclusion can be accepted, however, only when it is shown that a positive Gmelin test is certain proof of the presence of bilirubin.

Whipple and Hooper,[6] working along the same line on dogs, reported that, after cutting off all the blood supply to the liver, hemoglobin injected into the animal is promptly transformed into bile pigment. Their experiments, which have been widely quoted, were generally accepted as proving that the liver is not necessary for bilirubin formation. Rich,[7] however, has shown that the method employed by Whipple and Hooper does not shut off, but in fact permits, a very active circulation in the liver. In his own experiments, in which he either extirpated the liver, or completely cut off its circulation, there was not a trace of bilirubin in the blood serum, nor the slightest evidence of jaundice, in from one to five hours after the injection of hemoglobin.

2. Rous, P., and McMaster, P. D.: J. Exper. Med. **37**:11 (Jan.) 1923. Rous, P.; McMaster, P. D., and Broun, G. O.: J. Exper. Med. **37**:733 (June) 1923; ibid. **37**:395 (March) 1923.

3. Minkowski and Naunyn: Ikterus durch Polycholie, Arch. f. exper. Path. u. Pharmakol. **21**:1, 1886.

4. McNee, J. W.: Med. Klin. **28**:1125, 1913; J. Path. & Bacteriol. **18**:325, 1913.

5. Jones, C. M., and Jones, B. B.: Study of the Hemoglobin Metabolism in Paroxysmal Hemoglobinuria, Arch. Int. Med. **29**:669 (May) 1922.

6. Whipple, G. H., and Hooper, C. W.: J. Exper Med. **17**:612, 1913; Am. J. Physiol. **42**:256 (Jan.) 1917.

7. Rich, A. R.: Bull. Johns Hopkins Hosp. **34**:321 (Oct.) 1923.

Opposed to these experiments of Rich are some reported by Mann.[8] The latter has devised a three stage operation whereby the entire liver may be removed without disturbing the general circulation, and allowing a postoperative period of from two to ten hours, during which time the animal appears normal. Mann states that, within a few hours after hepatectomy, bile pigment, or a substance responding to the tests for bile pigment, appears in the plasma, tissues and urine. He offers no suggestion as to the origin of this substance.

We are unable to explain the difference in results between these two sets of experiments. Mann states that the pigment begins to appear in the urine a few hours after the removal of the liver, whereas Rich's maximum time of experiment was five and a half hours. It is possible that, some hours after removal of the liver, other tissues may become effective as bile formers. Again, there is a possibility of bile escaping into the abdominal cavity during the operation and being later absorbed. Neither explanation seems plausible. We feel, however, that while the question of place of formation of bile pigments is still an open one, the weight of available evidence places the liver as the chief, if not the only, organ concerned in the final process.

Whether or not the liver is the only organ capable of forming bile pigments, the pigments themselves normally eventually pass through this organ into the intestine. The amount of bilirubin in blood serum is minute, one-two hundred fifty thousandths, and its presence can be explained either as an overflow from the liver, from a formation elsewhere, or from an absorption from the small intestine. The daily flow of bile, under suitable experimental conditions, is quite regular. The liver has a large factor of safety in regard to bile excretion, and McMaster and Rous [9] have shown that an obstruction corresponding to three fourths of the bile ducts may be brought about without the appearance of jaundice.

The bile pigments entering the intestine undergo a gradual transformation on reaching the colon. It has recently been denied that any of the bilirubin is reabsorbed as such. In 1868, Jaffe [10] isolated from the urine and bile a substance with definite spectroscopic properties, which he called urobilin. He also was able to show that this substance showed a characteristic chemical reaction, namely, a green fluorescence, when an alkaline solution was treated with zinc chlorid. Vanlair and

8. Mann, F. C., and Magath, T. B.: Effect of Total Removal of Liver, Collected Papers of the Mayo Clinic **13**:200, 1921.

9. McMaster, P. D., and Rous, P.: J. Exper. Med. **33**:731 (June) 1921.

10. Jaffe: Beitrag zur Kenntnis der Galeen- und Harnpigmente, Zentralbl. f. med. Wissensch., 1868, p. 241; Ueber Fluoreszenz des Harnfarbstoffes, ibid. 1869, p. 177.

Masius,[11] in 1871, obtained from the stool a pigment, stercobilin, which they considered differed slightly from urobilin in spectroscopic relations. Maly,[12] at about the same time, succeeded in obtaining from bilirubin, through reduction with sodium amalgam, a pigment corresponding spectroscopically and in chemical reaction to urobilin. He called this pigment hydrobilirubin. Somewhat later, Müller demonstrated that bilirubin, in hydrogen atmosphere, was transformed by intestinal bacteria into hydrobilirubin. Finally, Garrod and Hopkins [13] showed that urobilin from normal and pathologic urines, from feces, and from bile removed from the gallbladder postmortem is one and the same thing. More recently, Hans Fischer and Meyer-Betz [14] and Charnas [15] again took up the study of urobilogen. They were able to isolate it from pathologic urines and feces, and proved its identity with hemibilirubin, which Fischer obtained in a 50 per cent. yield by reducing bilirubin with sodium amalgam.

Jaffe had found that the urobilin content in urine increased on exposure to light and air. Disqué [16] was later able to show that, by strong reduction of bilirubin with sodium amalgam and other reducing agents, the chromogen of urobilin, urobilogen, could be obtained. By subsequent oxidation, this could be changed to urobilin.

The chemical structure of urobilogen, from the work of Nencki,[17] contains a pyrrol nucleus. Hans Fischer [18] later established the chemical formula of $C_{33}H_{42}O_6N_4$. According to Fischer and Roese,[19] it has four pyrrol nuclei in common with hemin and bilirubin. Neubauer,[20] in 1903, showed that Ehrlich's reagent, dimethylamidobenzaldehyd, in hydrochloric acid solution, gives a typical red coloration of urobilogen solutions. This reaction is one common to pyrrol groups.

### ORIGIN OF UROBILOGEN

Since the publication of Friedrich Müller on the subject, the enterogenous origin of urobilogen has been generally accepted. Briefly

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11. Vanlair and Masius: Ueber einen neuen Abkommling des Gallenfarbstoffes im Darminhalt, Zentralbl. f. med. Wissensch., 1871, p. 369.
12. Maly: Kunstliche Umwandlung von Bilirubin in Harnfarbstoff, Zentralbl. f. med. Wissensch., 1871, p. 849.
13. Garrod and Hopkins: Preparation of Urobilin, J. Physiol. **20**:112, 1896; ibid. **22**:451, 1898.
14. Fischer, H., and Meyer-Betz, F.: Ztschr. f. physiol. Chem. **75**:232, 1911; ibid. **75**:339, 1911. München. med. Wchnschr. **59**:799, 1912.
15. Charnas: Biochem. Ztschr. **20**:401, 1909.
16. Disqué: Ueber Urobilin, Ztschr. f. physiol. Chem. **2**:1877, 1878.
17. Nencki and Zaleski: Berl. Berichte **34**:997, 1901.
18. Fischer, Hans: Ztschr. f. physiol. Chem. **73**:204, 1911.
19. Fischer and Roese: Ztschr. f. physiol. Chem. **89**:25, 1914.
20. Neubauer, Otto: Ueber die neue Ehrlichsche Reaktion mit Dimethylamidobenzaldehyde, Sitzungsb. d. Gesellsch. f. Morphol. u. Physiol. in München **32**, 1903.

stated, this conception is as follows: Bilirubin, entering the intestine, undergoes gradual changes, and, eventually, in the large intestine, by means of bacterial decomposition, becomes transformed into urobilogen. Maly was first to call attention to the action of bacteria on bilirubin. Later, Friedrich Müller [21] obtained urobilogen by bringing bilirubin in contact with peptone solution and putrefactive bacteria. Beck [22] obtained similar findings by mixing sterile bile with various strains of microorganisms. The urobilogen thus formed in the intestine is eliminated, in the main, with the feces. Some of it undergoes intestinal absorption and is carried to the liver, there to undergo further changes; part of it, however, is eliminated in the urine unchanged. A striking support of this conception is afforded by a case reported by Müller. He administered to a patient, with a complete biliary obstruction and with no traces of urobilogen in the urine, large quantities of bile. Within twenty-four hours, urobilogen appeared in the feces, and within forty-eight hours in the urine. Further support of this view is the fact that in the first days of the new-born, no urobilogen is found in the meconium, also that, in active purgation, the urobilogen disappears and unchanged bile pigment is found in the feces.

There has been some opposition to the views of Müller. In this country, Whipple and Hooper,[1] particularly, have questioned the intestinal production of urobilogen and its absorption into the portal circulation. In fact, only as recently as 1922, they stated, "There is not a shred of evidence, chemically or experimentally, to indicate that stercobilin is ever absorbed from the intestine." And again, "The intestinal absorption of stercobilin should be discredited until some positive evidence of such absorption is brought forward."

Ladage,[23] after the administration of bilirubin by mouth, obtained a marked increase in urobilin excretion in the stool, and a slight but definite increase in the urine. On giving 100 mg. of urobilin by mouth, he obtained a marked increase of urobilogen in the urine. It seems to us that this is positive evidence of intestinal absorption of the substance.

In dogs with biliary fistula and common duct occlusion, Fischler [24] has found urobilogen in the bile coming through the fistula. Wilbur and Addis [25] have reported similar findings in animals as well as in men

21. Müller, Friedrich: Ztschr. f. klin. Med. **12**:45, 1887; Verhandl. Kong. f. inn. Med., 1892, p. 118.

22. Beck, A.: Ueber die Entstehung des Urobilins, Wien. klin. Wchnschr. **35**, 1895.

23. Ladage, A. A.: Bijdrage tot de kennis de Urobilinurie, Dissertation, Leyden, 1899, quoted by Fischler (Footnote 24).

24. Fischler, F.: Physiologie und Pathologie der Leber, 1906.

25. Wilbur, R. L., and Addis, T.: Urobilin: Its Clinical Significance, Arch. Int. Med. **13**:235 (Feb.) 1914.

with cholecystostomy operations. Schneider,[26] and Jones,[27] working with duodenal bile, also have found urobilogen. Jones[5] reports an increase in the urobilogen of the duodenal bile in a case of paroxysmal hemoglobinuria within only a few hours after the production of an intravascular hemolysis of a peripheral vessel. These workers, as well as Hayem of the French school, believe in an extra-enterogenous origin of urobilin, and that the liver itself possesses the faculty of forming this substance.

Considering, therefore, these conflicting views, a series of experiments were undertaken by us, which are herein reported. We found, on the whole, no evidence pointing to an extra-enterogenous formation of urobilogen. In the main, our experiments substantiate Müller's conception. We feel that this fact is an important one since the usefulness of the urobilogen test, as an evidence of liver disturbance and as an aid in the clinical differentiation between various types of icterus, depends entirely on the enterogenous origin of urobilogen.

It is quite possible to explain the presence of urobilogen in the liver bile, as we shall see later, as the result of an infection in the biliary passages on the one hand, and, on the other, as a return excretion of the absorbed intestinal urobilogen.

The belief that urobilogen may be formed in the kidney, through a reduction occurring in that organ, appears to have been definitely disproved.

## METHODS OF ESTIMATION

The methods commonly used to determine the presence of urobilogen and urobilin are: (1) fluorescence in the presence of zinc salts, (2) spectroscopic absorption bands, (3) production of a red color by the addition of Ehrlich's aldehyd reagent.

(1) The fluorescent test is a test for urobilin only. While the fluorescence of urobilin, in the presence of zinc salts, was shown by Jaffe, in 1868, yet its practical application as a test is due to Schlesinger,[28] who, in 1903, introduced the saturated alcoholic solution of zinc acetate. This, in a slightly alkaline reaction, gives a green fluorescence with urobilin. The test is a very delicate one. In working with pure solutions of urobilin, he could obtain reactions up to 0.002 per cent. (1:50,000 dilution). Fischler, in 1906, applied this test to the quantitative estimation of urobilin, after having converted the urobilogen to urobilin by direct exposure to sunlight. In comparing the delicacy of this test to the spectroscopic disappearance of the absorption bands, he found it twenty-two and one-half times as sensitive (0.002 per cent. as compared to 0.045 per cent.).

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26. Schneider, J. P.: Anemia, Arch. Int. Med. **17**:32 (Jan.) 1916.

27. Jones, C. M.: Blood Pigment Metabolism and Its Relation to Liver Function, Arch. Int. Med. **29**:643 (May) 1922.

28. Schlesinger, H.: Zum klinischen Nachweis des Urobilins, Deutsch. med. Wschnschr. **32**:561, 1903.

The quantitative fluorescence test was later simplified by Marcussen and Hansen[29] and again by Adler[30] who converted the urobilogen into urobilin by the addition of a three per cent. solution of iodine, according to Ladage,[28] and by Conner and Roper.[31] Adler employed the saturated alcoholic solution of zinc acetate to carry on his dilutions. He calculates his final results in terms of milligram of urobilin.

(2) The spectroscopic method dates back to Gerhardt,[32] who, in 1889, used the spectrophotometer, employing Vierordt's tables to study the light extinction of solutions of urobilin as compared to normal urine. Since then, the spectroscopic method has had a very wide application, being used also in the Charnas[18] and Brugsch and Retzlaff[33] methods of urobilogen and urobilin estimations.

Wilbur and Addis,[25] in this country, in 1914, introduced a very practical quantitative spectroscopic method for estimating the combined urobilogen and urobilin. They make use of Charnas' principle of reading the urobilogen after the addition of Ehrlich's reagent, estimating at the same time the urobilin in the acid solution, which gives high readings and a better constant. The test is performed as follows: To 10 c.c. of urine out of a twenty-four hour specimen is added 10 c.c. of a saturated alcoholic solution of zinc acetate; this is filtered. To 10 c.c. of the filtrate, 1 c.c. of Ehrlich's reagent is added. The spectroscopic reading is made anywhere from fifteen minutes to an hour afterward. "The filtrate is gradually diluted with tap water until first one and then the other bands of light absorption have disappeared when the full amount of light enters the spectroscope, but are still visible when the light is partly shut off." The dilutions required give the value for 5 c.c. of urine. Multiplying this figure by the number of 5 c.c. in the total twenty-four hour urine, one obtains the total figure of urobilogen and urobilin in the twenty-four hour specimen.

(3) Ehrlich's aldehyd reaction, which consists of a red condensation product was first introduced by Otto Neubauer, in 1903. The reagent is composed of 2 gm. of paradimethylamidobenzaldehyd in 100 c.c. of 20 per cent. hydrochloric acid solution. This test for urobilogen is very sensitive. According to Fischer, it will still give a reaction with a dilution of one part of urobilogen to 640,000 parts of water.

Brugsch and Retzlaff, in 1912, and Flatow and Brunnell, in 1913, attempted quantitative estimations by using colorimetric standards, one employing a Bordeaux red solution, and the latter, phenolphthalein.

### AUTHORS' METHOD

The quantitative test devised by the authors is dependent on Ehrlich's aldehyd reaction, and consists of a series of dilutions of the urine carried to a point where no further reaction takes place. The end-result is read off where the faintest pink is still discernible. The reading is made by looking through the mouth of the test tube, holding the tube obliquely against a white background. Dilutions may be made with plain tap water.

Following are the details of the method: First 1 c.c. of Ehrlich's reagent is added to the whole urine, and the strength of the reaction

29. Marcussen and Hansen, S.: J. Biol. Chem. **36**:381 (Nov.) 1918.
30. Adler, A.: Deutsch. Arch. f. klin. Med. **138**:309 (Feb.) 1922; ibid. **140**: 302 (Nov.) 1922.
31. Conner, L. A., and Roper, J. C.: The Relations Existing Between Bilirubinemia, Urobilinuria and Urobilinemia, Arch. Int. Med. **2**:532 (Jan.) 1909.
32. Gerhardt, C.: Ueber Urobilinurie, Wien. med. Wchnschr. **27**:577, 1877.
33. Brugsch and Retzlaff: Ztschr. f. exper. Path. u. Therap. **11**:508, 1912.

noted by the rapidity and intensity of its development. The full development requires from one to three minutes. In strong urobilogen concentrations, a deep red color comes on promptly. One learns quickly to gage the presence of abnormal quantities from this qualitative test, and dilutions are not carried out if the color remains a light red within the time allowed, as here one deals with normal values. If, however, the original reaction indicates an increase, we then make a series of dilutions by adding 1 c.c. of the urine to 20, 30, 40, 50, 100, 200 c.c. of water, or more. From 10 to 15 c.c. of each dilution is placed in test tubes, and to each is added 1 c.c. of Ehrlich's reagent. The reading is made after from three to five minutes so as to allow the full development of the color. The last dilution must be the faintest pink discoloration, and the quantitative determination is expressed in terms of the greatest dilution of the urine in which the pink color is present, 1 : 10, 1 : 50, etc. If the color does not appear within five minutes, it may be disregarded. If the tap water is too cold, the reaction may be somewhat retarded. Care should be taken that the color is a genuine pink and not a yellowish brown, as is often found in urines containing bile or in concentrated urines. The test is preferably carried out in daylight. It is best not to make a reading in the bright sun as the shining rays have a tendency to intensify the color. Artificial light also does this to some extent.

In carrying out the test, we have disregarded entirely the estimation of urobilin, as this substance is never found in the freshly voided urine and forms only very slowly if the urine is kept away from the sunlight. We have made repeated observations on the daily loss of urobilogen, and have found that, if we allowed urines containing known amounts of urobilogen to stand for twenty-four hours, away from the strong light and in the cold, there was a loss, after twenty-four hours, of one-fifth the total urobilogen. If the exposure was continued for another twenty-four hours, there was a loss of another fifth, and so on, approximating a fifth or slightly less for every twenty-four hours.

We prefer to examine single fresh specimens rather than total twenty-four hour specimens, as we may thus find at least one specimen with a high urobilogen content. Such increase, if occurring even once a day, signifies an existing pathologic condition.

### ADVANTAGES OF METHOD

In comparing our method with the Wilbur and Addis spectroscopic method as to delicacy and final end-results, we found that we could carry our dilution five or six times as high as the disappearance of the spectroscopic bands would indicate. Through the courtesy of Dr. Gettler of Bellevue Hospital, comparisons between our test and the Wilbur-Addis test were made. In one urine, for instance, it was found that the spec-

troscopic bands were still visible at a 1:30 dilution and were totally absent at a 1:40 dilution. (This would mean a 1:60 dilution, as only half the original solution was urine.) In our test, however, the dilution could be carried to 1:350 before the final color disappeared, showing our test to be six times as delicate.

The quantitative fluorescent test possesses several elements of uncertainty: (*a*) the variability of the fluorescence, the intensity varying with fine shades of differences in the reaction, thus often interfering with the end-result; (*b*) the conversion of urobilogen into urobilin by oxidizing agents such as iodin or sunlight, wherein uncertainty arises as to whether the entire urobilogen has been thus changed. The sunlight always causes some loss in the urobilin. (*c*) Some loss in the urobilin when present in large amounts also takes place in the precipitation with the alcoholic solution of zinc acetate. (*d*) Special light is required in reading the final dilutions.

Our test also is more delicate than the fluorescent test. It is very simple, and does not require any complicated chemical procedures or special apparatus. It requires only a few minutes for its completion and can be done in any office, irrespective of the kind of light.

The examination of urobilogen in the stool presents difficulties due to interferences from indol and skatol. These substances give a similar reaction with Ehrlich's aldehyd reagent, and show similar spectroscopic absorption bands in alcoholic extractions. It is therefore necessary first to remove these putrefactive substances. This is best done by rubbing the stool in a mortar with petroleum ether and centrifugating. Several such extractions are made until the clear supernating petroleum ether no longer gives the Ehrlich's reaction. Of the remaining feces, alcoholic extractions are made until all the urobilogen is taken up. Using a definite weight of feces and a definite amount of alcohol, the urobilogen can be quantitatively estimated in similar dilutions.

### ANIMAL EXPERIMENTS

Our experiments were undertaken with the objects: (1) to make clear to ourselves the significance of an increase in urobilogen, particularly the relation of liver damage to urobilogen, and (2) to study the rôle of the intestine in the formation of urobilogen, i. e., the necessity of a flow of bile into the intestine for its formation, particularly in view of the many different opinions as to the mode and place of origin of this substance. We have repeated some of Fischler's experiments and added others of our own.

Female dogs were used for this purpose, the urine being obtained by catheterization and examined for urobilogen immediately after with-

drawal. In the normal dog, urobilogen appears in the minutest traces and the Ehrlich's aldehyd reaction gives a faint pink color. In a dilution of 1 : 10 the result is usually negative.

To produce the liver damage, we have used a number of known liver poisons, but have confined ourselves especially to chloroform because of its certainty of action.[34]

After a preliminary period of observation, during which, in some experiments, food was withheld from the animals for a few days,[35] chloroform was given by inhalation, in an amount sufficient to induce surgical anesthesia, and continued over a period of from one half to two hours (usually between one and one half and two). The urine was examined at intervals during the following days. In some of these experiments, a phenoltetrachlorphthalein test was made while the chloroform effects were present, to serve as a check and allow of a comparison of the two tests. The dye was injected intravenously, 5 mg. per kilogram of body weight, and the amount remaining in the serum determined at the end of one hour.[36] We also have made a few determinations of the effects of carbon tetrachlorid on urobilogen excretion. This substance has been shown, by Lamson,[37] to be toxic to the liver and to affect markedly phenoltetrachlorphthalein excretion. Some typical protocols are given in Table 1.

## DISCUSSION OF EXPERIMENTAL RESULTS

The experimental results we have cited show that whenever the liver is damaged by chloroform there results a decided rise in the urinary urobilogen. The rise in terms of dilution may vary from 1 : 50 to 1 : 1,000, and is undoubtedly in proportion to the degree of liver damage. When carbontetrachlorid is administered prior to the chloroform, the effect is intensified. The urobilogen rise commonly occurs on the day following the chloroform inhalation, although, in some instances, it may be delayed or require several inhalations before the damage becomes evident. The return to the normal occurs abruptly, from which the inference may be drawn that the functional activity, once started, becomes restored with great promptness.

In the experiments with carbontetrachlorid, in Dogs 3 and 5, both the urobilogen and phenoltetrachlorphthalein test were used simulta-

34. Davis, N. C., and Whipple, G. H.: Influence of Drugs and Chemical Agents on Liver Necrosis of Chloroform Anesthesia, Arch. Int. Med. **23**:636 (May) 1919. Whipple, G. H., and Speed: J. Biol. Chem. **18**:447, 1914.

35. Whipple, G. H., and Speed: J. Biol. Chem. **18**:612, 1914.

36. Rosenthal, S. M.: J. Pharm. & Exper. Therap. **19**:385 (June) 1922; New Method of Testing Liver Function with Phenoltetrachlorphthalein, J. A. M. A. **79**:2151 (Dec. 23) 1922.

37. Lamson, P. D., and McLean, A. J.: Toxicity of Carbontetrachlorid, J. Pharm. & Exper. Therap. **31**:237 (May) 1923.

neously in order to determine how these two tests compare with the same liver damage. We find that the results of the two tests are not parallel, and that the liver function appears to be altered more in respect to the dye excretion than as regards the urobilogen.

TABLE 1.—*Typical Protocols*

| | Date | Chloroform Carbontetrachlorid | Urobilogen in Urine | Bile in Urine | Phenoltetrachlorphthalein |
|---|---|---|---|---|---|
| Dog 8 | Jan. 16 | | Traces | ..... | ..... |
| | Jan. 17 | Carbontetrachlorid, 50 c.c. | Traces | ..... | ..... |
| | Jan. 18 | Chloroform, 1 hour 50 minutes | Traces | ..... | ..... |
| | Jan. 19 | | Traces | ..... | ..... |
| | Jan. 21 | Chloroform, 1½ hours | 1:30 | ..... | ..... |
| | Jan. 22 | | 1:100 | ..... | ..... |
| | Jan. 23 | | 1:1,000 | ++ | ..... |
| | Jan. 24 | | 1:500 | + | ..... |
| | Jan. 25 | | 1:10 | ..... | ..... |
| Dog 10 | March 1 | | Traces | ..... | ..... |
| | March 2 | Chloroform, 1¾ hours | Traces | ..... | ..... |
| | March 3 | | 1:150 | ..... | ..... |
| | March 4 | | 1:75 | ..... | ..... |
| | March 5 | | Traces | ..... | ..... |
| Dog 7 | Jan. 29 | Chloroform, 1¾ hours | Traces | ..... | ..... |
| | Jan. 30 | | 1:50 | ..... | ..... |
| | Jan. 31 | | 1:75 | ..... | ..... |
| | Feb. 1 | | Traces | ..... | ..... |
| Dog 5 | Dec. 10 | | Traces | ..... | ..... |
| | Dec. 11 | Carbontetrachlorid, 75 c.c. | Traces | ..... | ..... |
| | Dec. 12 | | 1:25 | ..... | ..... |
| | Dec. 13 | Carbontetrachlorid, 50 c.c. | Traces | + | ..... |
| | Dec. 14 | | 1:30 | ..... | 7% after 1 hour |
| | Dec. 15 | | 1:30 | ..... | ..... |
| | Dec. 19 | Chloroform, 2 hours | 1:30 | ..... | 1% after 1 hour |
| | Dec. 20 | | 1:300 | ++ | 4% after 1 hour |
| | Dec. 21 | | 1:250 | + | ..... |
| | Dec. 22 | | 1:100 | + | ..... |
| | Dec. 23 | | 1:75 | + | ..... |
| | Dec. 24 | | 1:50 | ..... | ..... |
| | Dec. 25 | | 1:30 | ..... | ..... |
| | Dec. 28 | Chloroform, 1½ hours | Traces | ..... | ..... |
| | Dec. 29 | | 1:50 | + | ..... |
| | Dec. 30 | | 1:40 | ..... | ..... |
| | Dec. 31 | | 1:50 | ..... | ..... |
| | Jan. 2 | | Traces | ..... | ..... |
| Dog 6 | Dec. 24 | | Traces | ..... | ..... |
| | Dec. 26 | Chloroform, 2 hours | Traces | ..... | ..... |
| | Dec. 27 | Chloroform, 2 hours | 1:60 | + | ..... |
| | Dec. 28 | | 1:50 | + | 10% after 1 hour |
| | Dec. 29 | | 1:50 | + | ..... |
| | Dec. 30 | | 1:100 | ..... | ..... |
| | Dec. 31 | | Traces | ..... | ..... |
| Dog 4 | Dec. 3 | | Traces | ..... | ..... |
| | Dec. 4 | Chloroform, 2 hours | Traces | ..... | ..... |
| | Dec. 5 | Chloroform, ½ hour | Traces | ..... | ..... |
| | Dec. 6 | Chloroform, ½ hour | 1:35 | ..... | ..... |
| | Dec. 7 | | 1:75 | ..... | 6% after 1 hour |
| | Dec. 8 | | 1:50 | ..... | ..... |
| | Dec. 9 | | Traces | ..... | ..... |
| | Dec. 10 | Chloroform, 1 hour | Traces | ..... | ..... |
| | Dec. 11 | Chloroform, 1¾ hours | Traces | ..... | ..... |
| | Dec. 13 | | 1:125 | ..... | ..... |
| | Dec. 14 | | Traces | ..... | ..... |

Somewhat similar quantitative differences are seen when the liver is damaged with chloroform. The evidence presented points then to these two tests as being supplementary and not indicative of a single functional disturbance. While our experiments are not conclusive on this

point, we feel that the purely excretory function shown by the phenoltetrachlorphthalein test differs from that relating to the urobilogen. Similar differences will also be shown in our clinical material.

### LIGATION AND EXCISION OF THE COMMON BILE DUCT

After having established the relation between liver damage and the urobilogen output, with a normal flow of bile into the intestine, we then took up the second point for investigation, which concerns the rôle of the intestine in the formation of urobilogen, or the dependence of the urinary urobilogen on the flow of bile into the intestine. In determining this point, we have ligated the common bile duct in the animals that had been previously tested out as to the effect of chloroform anesthesia on the output of urobilogen. We have used the dogs reported in the preceding experiments after their urinary urobilogen had returned to normal. The common bile duct was ligated in two places and a segment of the duct was resected between the two ligatures. In Dog 8, a biliary fistula also was made. After the animals had sufficiently recovered from the operative procedure, chloroform was again administered, and the effects noted. An anastomosis between the gallbladder and duodenum was made in Dog 7, ten days after the ligation and excision of the common duct. Fresh pig's bile was administered by mouth to Dog 5, the second day after the ligation of the common duct, and continued for several days. The protocols of the experiments are given in Table 2.

We feel that the experiments given in Table 2 clearly substantiate the intestinal formation of urobilogen, and show that without the presence of bile in the intestine, urobilogen is not formed, even though the liver is subjected to the same degree of damage as before. As is seen in Dogs 10 and 6, after the ligation of the common duct, repeated chloroform administrations do not cause the appearance of any urobilogen. In Dog 7, when the flow of bile was reestablished in the intestine by an anastomosis between the gallbladder and the duodenum, the urinary urobilogen promptly made its appearance. Similarly, in Dog 5, when bile was fed by mouth, the urobilogen also appeared in the urine.

In this connection, we wish to cite a case which presents some analogy to our experimental work on Dog 7. A patient, aged 40, developed jaundice, in December, 1923. The jaundice increased, becoming intense in January, with an acholic stool and evidence of complete obstruction of the bile duct. Urobilogen, at this time, was totally absent from the urine. In February, an exploratory operation was performed and a hard mass found infiltrating the head of the pancreas, the gallbladder being dilated. An anastomosis between the gallbladder and stomach was made. A week after the operation, the jaundice had greatly diminished, and the stools became brown. The urobilogen was positive in a 1:40 dilution.

Only in Dog 8, with a gallbladder fistula, in which a severe infection took place spreading throughout the liver and later to the other abdominal organs, did the urinary urobilogen rise after chloroform anesthesia. The appearance of urobilogen, in this dog, with an occlusion of the common duct might cause one to believe in the hepatic origin of the substance. On closer study, however, it can be explained on the basis of an ascending infection along the biliary radicles. The same process of decom-

TABLE 2.—*Protocols of Experiments in Which Ligation and Excision of Common Bile Duct Was Done*

| | Date | Chloroform, Carbontetrachlorid | Urobilogen in Urine | Bile in Urine |
|---|---|---|---|---|
| Dog 10 | March 2 | Chloroform, 1¾ hours | Traces | ..... |
| | March 3 | ..... | 1:150 | ..... |
| | March 10 | Common bile duct ligated | Traces | ..... |
| | March 12 | ..... | 0 | ++++ |
| | March 13 | Chloroform, 1½ hours | 0 | ++++ |
| | March 14 | Chloroform, 1¾ hours | 0 | ++++ |
| | March 15 | ..... | 0 | ++++ |
| Dog 7 | Jan. 29 | Chloroform, 1¾ hours | Traces | ..... |
| | Jan. 30 | ..... | 1:75 | ..... |
| | Feb. 15 | Bile duct ligated and excised, also biliary fistula | Traces | ..... |
| | Feb. 19 | ..... | 0 | ..... |
| | Feb. 20 | Chloroform, 1¾ hours | 0 | ..... |
| | Feb. 21 | ..... | 0 | ++ |
| | Feb. 23 | ..... | 0 | ++++ |
| | Feb. 25 | Anastomosis between gallbladder and duodenum | 0 | ++++ |
| | Feb. 27 | ..... | 1:40 | +++ |
| Dog 6 | Dec. 30 | After chloroform, 2 hours | 1:100 | ..... |
| | Dec. 31 | ..... | Traces | ..... |
| | Jan. 2 | Bile duct ligated and excised | Traces | ..... |
| | Jan. 3 | ..... | 1:40 | ..... |
| | Jan. 5 | Chloroform, 1½ hours | 0 | ..... |
| | Jan. 6 | ..... | 0 | ..... |
| | Jan. 7 | Chloroform, 1¼ hours | 0 | ..... |
| | Jan. 8 | Chloroform, 1 hour | 0 | ..... |
| Dog 5 | Dec. 25 | After chloroform | 1:300 | ..... |
| | Jan. 2 | Common bile duct ligated and resected | Traces | ..... |
| | Jan. 3 | 75 c.c. fresh pig's bile by mouth | ..... | ..... |
| | Jan. 4 | 50 c.c. fresh pig's bile by mouth | ..... | ..... |
| | Jan. 5 | ..... | 1:25 | ..... |
| Dog 8 | Jan. 23 | After chloroform | 1:1,000 | ..... |
| | Jan. 30 | Common duct ligated and excised, gallbladder fistula | Traces | ..... |
| | Jan. 31 | ..... | 0 | ..... |
| | Feb. 1 | ..... | 0 | ..... |
| | Feb. 2 | ..... | 0 | ..... |
| | Feb. 3 | ..... | 0 | ..... |
| | Feb. 4 | ..... | 0 | ..... |
| | Feb. 5 | Chloroform, 1½ hours | 0 | ..... |
| | Feb. 6 | ..... | 1:50 | ..... |

position of bilirubin which takes place in the intestinal canal by the bacterial flora may take place in the liver substance itself, within the biliary radicles. Such urobilogen may then be excreted with the bile, and, if the parenchyma of the liver also is damaged following the prolonged chloroform anesthesia, then the urobilogen escapes into the general circulation.

In our animals with biliary fistula, no urobilogen could be found in the fistula bile within the first few days after the operation. Later, however, when infection took place, as in Dog 8, urobilogen made its

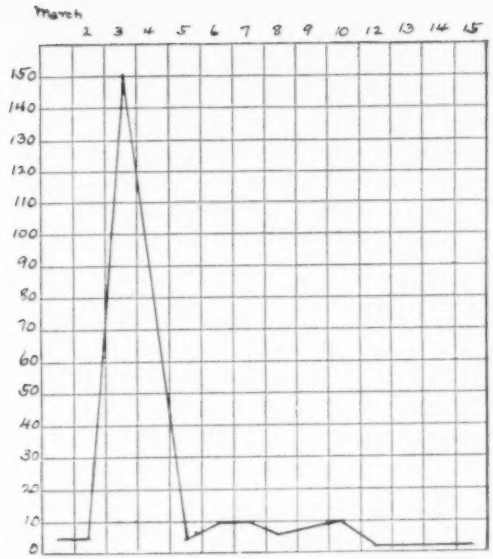


Chart 1. (Dog 10).—Urobilogen elimination in liver damage before and after exclusion of bile from intestine. The ordinates, in this and the following charts, represent the highest dilutions of the urine at which the urobilogen test was still positive. March 2, chloroform anesthesia was given for one and three-fourth hours; urobilogen rose from traces to 1:150 within twenty-four hours; it came down to normal within forty-eight hours. March 10, the common bile duct was ligated; urobilogen came down to zero, March 12. Chloroform was again administered, March 13, and repeated on the 14th; urobilogen remained at zero.

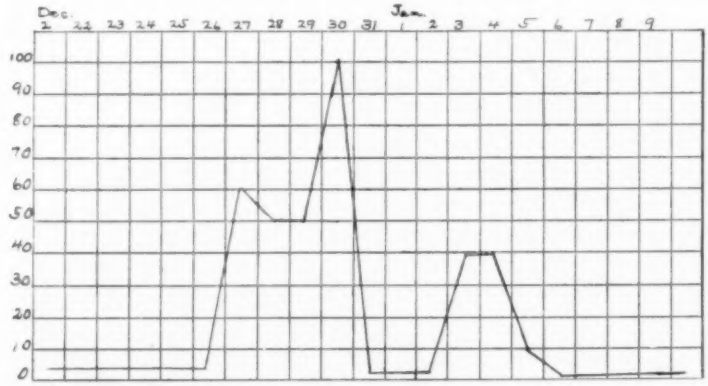


Chart 2 (Dog 6).—Similar effect to that shown in Chart 1 (Dog 10). December 26, chloroform anesthesia was given for two hours; urobilogen rose from traces to 1:60. December 27, chloroform was again repeated for two hours; urobilogen rose to 1:100, December 30, and fell to normal on the 31st. January 2, the bile duct was ligated and excised. There occurred a temporary rise of urobilogen lasting two days, probably due to the urobilogen content in the intestinal canal. Chloroform anesthesia was given January 5; urobilogen down to zero. Chloroform was repeated, January 7, and 8; urobilogen remained at zero.

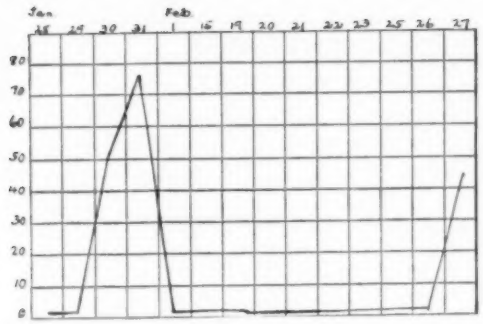


Chart 3 (Dog 7).—Urobilogen elimination in anastomosis between gallbladder and duodenum after exclusion of bile from intestine. January 29, chloroform anesthesia was given for one and three-fourth hours; urobilogen rose to 1:75 within two days, and fell to normal on the third day. February 15, the common bile duct was ligated. February 20, chloroform was administered for one and three-fourth hours; urobilogen fell to zero. February 25, anastomosis between gallbladder and duodenum was made; February 27, urobilogen rose to 1:45.

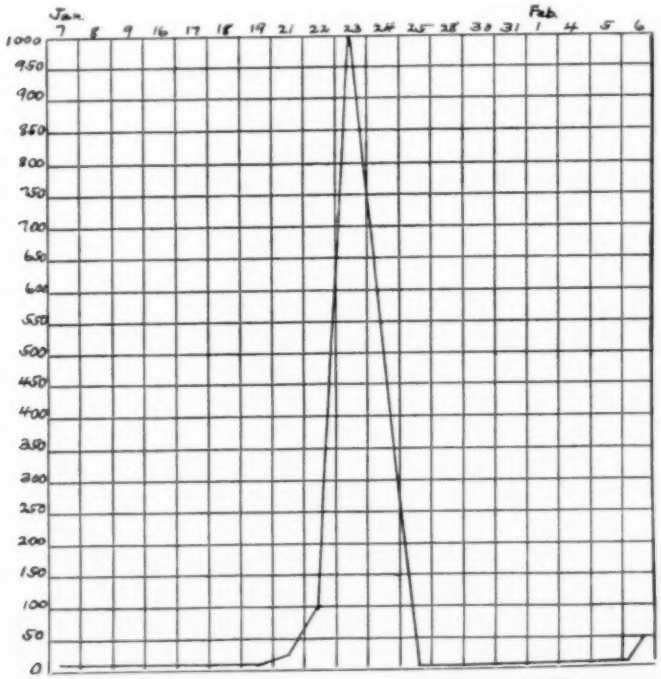


Chart 4 (Dog 8).—Urobilogen elimination in infection of biliary tract with exclusion of bile from intestine. Carbontetrachlorid and two sessions of chloroform were administered, between January 17 and 21; urobilogen rose, January 23, from traces to 1:1,000; it came down to normal, January 25. The common duct was ligated and a gallbladder fistula made, January 30; urobilogen fell to zero. There was severe infection of the liver and peritoneum. February 5, chloroform was given; urobilogen rose, on the 6th, to 1:50.

appearance. Dog A of Wilbur and Addis' experiments also illustrates that a low grade infection can occur through the biliary fistula, which may lead to pronounced liver changes. This dog with biliary fistula and common duct ligation eliminated large amounts of urobilogen. At postmortem examination, a marked cirrhosis of the liver was found

We have been unable to find any corroborative evidence in support of the formation of urobilogen by the liver parenchyma. The most striking phenomenon is the absence of urinary urobilogen in the animals with common duct ligation, in spite of the fact that the liver was subjected to considerable damage by repeated chloroform anesthesias. If urobilogen is a product of the liver cell itself, one should expect to find it in the urine in the cases with common duct ligation, in which all the secretions and excretions of the liver, being dammed back into the general circulation, have only one avenue of escape. Its absence from the urine speaks, therefore, against a hepatic origin.

### PRESENCE OF UROBILOGEN AND UROBILIN IN THE INTESTINE IN OCCLUSION OF THE COMMON DUCT

In examining the stools in common duct obstructions, we found, after careful extraction with petroleum ether, small quantities of urobilogen even after a preliminary flushing of the bowels with purgatives and cleansing enemas. We feel that the presence of these small quantities must be explained on the basis of an elimination of bilirubin from the blood serum into the intestine, and there changed to urobilogen. The scybala often presented a thin outer layer of dark brown which covered a central acholic portion. In making separate examinations of the central and outer layers, we found that the inner portion was entirely free from urobilogen and urobilin, but that the pigmented outer portion gave positive reactions. This tends to show, in these cases with saturation of bile in the plasma, that bilirubin is eliminated into the intestinal canal, coating the outer layer of the feces, and is there changed by bacterial putrefaction.

We cannot agree with Wilbur and Addis in the belief that these substances are formed in the serum and, as such, eliminated into the intestinal canal. The readiness with which urobilogen is excreted by the kidney is well known, and the question always arises why, in common duct obstruction, if urobilogen is formed in the blood stream, it is never found in the urine. The assumption, therefore, of an intestinal excretion is not plausible.

### CLINICAL CONSIDERATIONS

Urobilogen is a normal constituent of urine in man. It is present in smallest amount in the morning urine, or may be absent altogether. It rises somewhat in the afternoon and evening. In constipated individuals

with increased putrefactive changes in the large bowel, the quantity of urobilogen rises above the normal. Urobilin never appears in the freshly voided urine. Even in pathologic urines in which the urobilogen rises to abnormally high figures, urobilin is seldom, if ever, found, and when it is present it occurs in negligible traces.

Using our method of estimation, we found normally urobilogen in the morning urine in amounts which failed to give a reaction if the urine was diluted more than 1 : 10. During the afternoon and evening, the average maximum use of urobilogen was 1 : 20. Marcussen and Hansen,[37] using the fluorescence method, report similar findings.

In a series of more than 1,000 ambulatory patients, the routine examinations of the urine revealed, in more than 90 per cent. anywhere from traces to positive 1 : 20 dilutions. The remainder presented pathologic conditions relating to the biliary or intestinal tract, to the cardiovascular or hemopoietic system.

A group of 125 medical students also were examined. In seventy-five of these, specimens were obtained in the forenoon, and in the remaining fifty in the afternoon. Seventy-two of the forenoon urines were below 1 : 20, most of them being about 1 : 10. One was 1 : 25, one 1 : 50, and one was discovered with a very high urobilogen reading of 1 : 350. The afternoon group corresponded in the main with the morning one with the exception that the figures approached more nearly to 1 : 20. One of these showed 1 : 40 and one 1 : 50. Of the total, 96 per cent. gave readings below 1 : 20.

The elimination of urobilogen also presents in pathologic states great variations between the night and day output. It is always lowest during the night and early morning hours. During the day, the amounts may also vary, sometimes from hour to hour. Wilbur and Addis quote a case of bronze diabetes, which, while showing great irregularities in the output of urobilogen during the day, was always constant in eliminating the lowest quantities by night. If, however, this patient remained up and took his meals during the night and slept in the daytime, then the condition became reversed, the amount excreted during the night being much larger than during the day.

Saillet [38] suggests a relation of these variations to the process of digestion and general body metabolism. We may compare the mobilization of the urobilogen by night to the same process which takes place in a deficient kidney function, in which, during the night, the kidney continues to eliminate the excess of solids which it was incapable of eliminating during the day. So the liver during the resting period, when metabolic processes associated with digestion and general body activity are at a standstill, can attend to its retarded functions. The daily variations also must be explained on similar digestive and other

38. Saillet: Urobilin in Normal Urine, Rev. de méd. **17**:109, 1897.

functional interferences, the escape of the excess of urobilogen into the general circulation and into the urine always being highest in the afternoon when metabolic processes are at their highest. These variations are not dependent on changes in the excretory function of the kidney, and do not run parallel with changes in the specific gravity of the urine. Hourly observations of changes, often amounting to twice the amount of urobilogen, would show a variation of only one or two points in specific gravity, thus showing, furthermore, its independence of urine concentration.

### DIAGNOSTIC SIGNIFICANCE

Hildebrand was the first to note the numerous instances in which urobilogen appeared in the urine in increased amount. In fact, the frequency of this occurrence in a large variety of conditions has for the time detracted from its usefulness and has led even such observers as Friedrich Müller to express some doubt as to its diagnostic value. In later years, however, a great deal of clinical study has been made, particularly by Eppinger,[39] who grouped its pathologic occurrence as follows: (1) diseases of the liver, (2) hemolytic diseases, (3) cardiac diseases, (4) intoxications and (5) infectious diseases.

This grouping, however, may be reduced to two main classifications: (1) hepatic and biliary diseases, and (2) hemolytic diseases. All conditions in which urobilogen appears in increased amounts can be classed under one or the other of these two groups, or a combination of the two.

(1) *Diseases of the Liver and Biliary Tract.*—In this group are: catarrhal jaundice; cholangeitis; acute yellow atrophy; hepatic cirrhosis; chronic passive congestion of the liver associated with chronic endocarditis, especially in the state of chronic decompensation; liver abscess when the suppurative process is generalized but not a solitary lesion; miliary tuberculosis; primary carcinoma of the liver; metastasis producing a general carcinomatosis of the liver but not localized nodular areas; toxic states, such as eclampsia; toxic pregnancies and later stages of tuberculosis; intoxication resulting from the intake of large amounts of alcohol, and also infections such as scarlet fever and measles.

(2) *Hemolytic Diseases.*—Among these are grouped: hemolytic icterus; pernicious anemia; septic states; malaria, lead poisoning and other hemolysis produced by poisons such as antifebrin, sulphonal and mushrooms. Here it occurs as the result of marked increase in blood destruction with an increased pigment formation, associated also with liver involvement. Minot [40] has shown that 40 per cent. of pernicious anemia patients during life present a large liver. A pathologic state

39. Eppinger, Hans: Die Hepato-Lienalen Erkrankungen, 1920.
40. Minot, C. R.: Oxford Med. **2**:623, 1920.

of the liver can be demonstrated at necropsy in almost all cases presenting blood destruction, the liver changes often going hand in hand with the changes occurring in the spleen.

(1) DISEASES OF THE LIVER AND BILIARY TRACT

*Catarrhal Jaundice.*—The occurrence of urobilogen in excessive amounts is a constant phenomenon in catarrhal jaundice. While it is not present in all stages of the disease, as will be shown later, yet it invariably makes its appearance at one time or another during its course. This forms a marked distinguishing feature from the obstructive jaundice, in which the interference of the bile flow results from outside pressure on the common or hepatic ducts. In the obstructive jaundice conditions, urobilogen never appears in the urine.

Its occurrence in catarrhal jaundice indicates an acute parenchymatous change in the liver cells somewhat analogous to the changes produced by prolonged chloroform anesthesia. We cannot, therefore, regard the disease in the light of the old Virchow conception of an obstruction of the common duct due to a mucous plug. The flow of bile into the duodenum is always present. It may be scanty at times but it is never completely interrupted.

Out of a series of seventeen cases of catarrhal jaundice here reported, twelve were intubated with a duodenal tube. Bile was found in all cases, the pigment varying from scanty to normal amounts. The introduction of magnesium sulphate, according to the Meltzer-Lyon method, seldom yields, in these cases, the typical dark brown bile, the so-called B bile, as in the normal. There may occur a very faint increase in the intensity of the color, but it rarely becomes more viscid or turns to a deep brown. Microscopic examination made in six cases revealed, in each instance, the presence of pus cells, often in large clumps. This indicates the infectious character of the process.

Urobilogen was found in increased amounts in every case at the beginning of the disease and toward the end. During the height of the disease, it may be scanty or entirely absent, the duration of its absence depending on the severity of the disease. In the more severe cases, it may disappear for a few days at a time, and is due to the fact that very little bile enters the duodenum at that period.

If the patient is seen during the earlier stages of jaundice, the amount of urobilogen may be found anywhere up to a 1 : 80 or 1 : 100 dilution. This soon diminishes to small traces, or may become absent altogether for short periods. After the disease has lasted for several weeks, the urobilogen again makes its appearance in large amounts, and soon rises to high figures, anywhere up to a 1 : 250 dilution. The amounts vary daily. This stage may last as long as several weeks, presenting daily and often hourly variations. Its total disappearance indicates a restora-

tion of the liver function, even though a slight jaundice may still persist in the sclera or skin. The examination of the bilirubin in the serum at this stage also shows a return to normal. The slight staining of the tissues merely indicates an impregnation of the organs with bile pigment, which is very slowly given up.

The presence of urobilogen, even in traces, during the state of jaundice promptly rules out an obstruction or mechanical icterus, such as occurs in carcinoma of the head of the pancreas or of the biliary tract. This fact can be utilized to great advantage in the differential diagnosis between the jaundice due to hepatic causes and that due to extraneous mechanical causes. During the height of the disease, when urobilogen is absent from the urine, it may be somewhat difficult to differentiate between these two conditions. Repeated urine examinations, however,

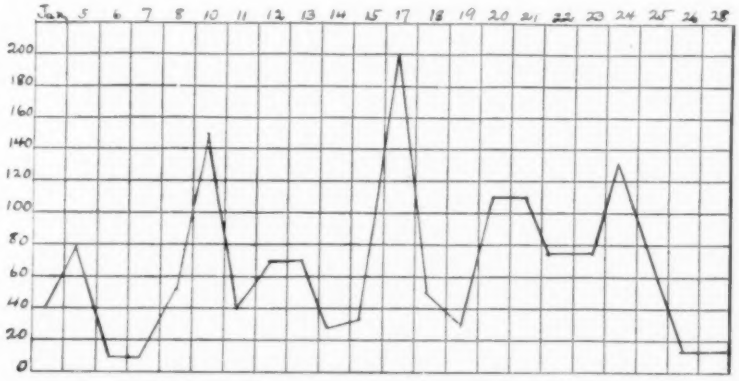


Chart 5.—Daily output of urobilogen during course of a case of catarrhal jaundice.

will invariably aid in the diagnosis. In the catarrhal jaundice, it will reappear within a few days. Its continued absence for a week or longer speaks for a mechanical jaundice due to malignant causes. A point which may be of considerable significance is the fact that in the mechanical jaundice, if Ehrlich's aldehyd reagent is added to the urine, it changes within a few minutes to an intense green. This reaction we have always noted when the obstruction is extreme. We have always found it in the series of our dogs with ligation and excision of the common duct.

*Septic Cholangeitis with Liver Abscess.*—Two cases are here reported, one following in the wake of an apparently simple catarrhal jaundice, the symptoms of cholangeitis coming on a few weeks after the patient had seemingly recovered from the disease. The patient suddenly developed an elevation of temperature with chills, the temperature

showing at times variations of ten degrees, between 97 and 107. Several weeks later, multiple abscesses were found on operation. The urobilogen was constantly present up to 1 : 200.

Another patient presented a history of digestive disturbances for some time prior to admission to the hospital. The patient ran similar high temperatures between 100 and 107 degrees, accompanied by severe daily chills and sweats. The urobilogen ran up to 1 : 500. After an illness of two months' duration, death occurred and the necropsy revealed multiple large and small abscesses in the liver.

*Urobilogen in Chronic Liver Affections.*—In chronic diseases, contrary to the acute states, one does not find an increase in the urobilogen as a constant factor. Many of the cases of hepatic cirrhosis here reported, with or without enlargement of the liver, with splenic enlargement and often ascites, failed to show pathologic increase in urobilogen. It has already been pointed out that a large factor of safety is presented by the liver regarding its functions, and how differently these diversified functions may be affected in disease. Only in grave pathologic conditions, such as acute yellow atrophy or phosphorus poisoning, may these functions be simultaneously involved.

That the liver is resistant to damage and can still perform its nitrogenous metabolism in spite of the fact that severe histologic changes can be demonstrated, has been shown by Howland and Richards,[41] who, in a study of the metabolism in dogs following severe and delayed chloroform poisoning, have found no interference with this function. McMaster and Rous[9] also have shown that, in the dog, nineteen-twentieths of the liver substance can be placed in a condition of stasis without the occurrence of tissue icterus. They find that such obstruction invariably results in atrophy of the affected tissue with compensatory hypertrophy elsewhere.

Our own observations point to the fact that the degree of interference with the liver function is dependent on the extent and rapidity of the disease process. In the slowly progressive liver affection, there appears to occur a gradual adaptation of the liver substance to carry on its function. The compensatory replacement of new cellular tissue is a well known characteristic of this organ. The occurrence of a large or fatty liver does not necessarily indicate a deficiency in its function. Neither does a chronically diseased liver with positive histologic changes always indicate a lack in the performance of its metabolic functions. Following are some of the findings in a series of chronic liver cases taken from the third medical division of Bellevue Hospital:

Case 1.—Cirrhosis of the liver, large spleen, marked anemia, ascites, hematemesis, no jaundice. Urobilogen, 1 : 10. February 4, blood transfusion.

41. Howland and Richards: Metabolism and Pathology of Late Chloroform Poisoning, J. Exper. Med. **11**:344, 1909.

February 5, urobilogen rose to 1:60. February 7 and 8, urobilogen came down to the previous low figures of 1:10, the increase in urobilogen being due to destroyed red blood cells from the transfused blood.

Case 2.—Banti's disease. Urobilogen, variation from 1:10 to 1:100.

Case 3.—Cirrhosis of the liver, marked anemia, ascites, jaundice of three months' standing. Urobilogen, 1:20.

Case 4.—Syphilitic liver. Urobilogen, 1:30.

Case 5.—Cirrhosis of the liver, jaundice, ascites, edema. Tapped; 5 liters removed. Urobilogen, 1:50, rose to 1:300 with increase in jaundice. Necropsy report was hypertrophic cirrhosis, spleen moderately enlarged.

Case 6.—Splenomegaly with anemia. Urobilogen, 1:30.

There were three cases of Hodgkin's disease. Urobilogen, normal.

In subacute conditions associated with various degrees of jaundice, the urobilogen is usually increased. These appear to follow attacks of catarrhal jaundice and finally lead to a hypertrophic or Hanot cirrhosis. They are characterized by persistence of jaundice lasting for months after the attack, accompanied by enlarged liver and spleen. With exacerbations in the course of the disease, the amount of urobilogen may rise to high figures. Two such cases have been under observation during the last year. Both patients were young adults about 30, non-alcoholic, with negative Wassermann reactions. In both, the disease had apparently developed in the wake of an attack of catarrhal jaundice. The urobilogen varied ordinarily between 1:30 and 1:40. During an exacerbation which took place in one of the patients, there occurred an aggravation of the symptoms, the jaundice became more intense, the liver somewhat tender, and the gastro-intestinal disturbances more acute. This condition lasted one month, during which time the urobilogen rose to 350, varying between 150 and 350, at the end of which period the jaundice gradually diminished to a slight icteric tint, the symptoms improved and the urobilogen came down to its previous level.

*Urobilogen in Cholecystitis and Cholelithiasis.*—In chronic cholecystitis and cholelithiasis, the urobilogen test showed no alteration in liver function in the majority of instances. Out of a series of 150 cases, nineteen showed a slight rise in the urobilogen output, varying from 1:35 to 1:50. The remainder gave normal readings during repeated examinations. In some of these cases, the bilirubin in the blood also was estimated, according to the Van den Bergh [42] method. The findings were likewise normal, the figure varying from 1:200,000 to 1:400,000.

Four cases of chronic hydrops of the gallbladder, with large tumor masses, gave normal urobilogen and bilirubin figures.

One case of acute cholecystitis and cholelithiasis with an elevation of temperature to 102 degrees and with severe colic requiring morphin, with

42. Van den Bergh, A. A.: Der Gallenfarbstoff im Blute, Leyden, 1918.

development of slight jaundice, probably indicating the passage of a stone through the common duct, gave a rise of urobilogen to 1 : 100, and bilirubin, 1 : 48,000.

In one case of chronic cholelithiasis associated with diabetes with a large liver, the patient showing a subicteric tint, the urobilogen was 1 : 50 and the bilirubin 1 : 110,000. Here the abnormal rise could be attributed to the chronic hepatic disease.

The foregoing findings therefore tend to indicate that in the chronic stage of biliary diseases, neither the urobilogen in the urine nor the bilirubin in the serum show any deviation from the normal in the majority of instances, and give us little or no aid in the diagnosis of these conditions. They are significant only in acute stages.

Recently, attention has been called by Heyd [43] and others to chronic histologic changes in the liver occurring in association with chronic gall-bladder disease. Histologically, they were able to show these changes by removing small sections of the liver during the course of operation. The report of their findings, in the chronic lesions, indicated structural liver changes simulating both the atrophic and hypertrophic cirrhoses. Heyd claimed that fatalities following operations on the biliary tract were due to unexplainable toxemias resulting from chronic liver degeneration. The study of the foregoing cases, however, does not seem to bear out the existence of a chronic liver derangement with any such disturbance in its metabolic function.

*Effect of Arsphenamin Therapy on Urobilogen Elimination.*—Fifty cases of syphilis from the Bellevue College Clinic were examined during the course of arsphenamin therapy. Most of these were old cases having received several courses of antisyphilitic treatment. Only two cases were in the secondary stage with the presence of roseola. Urinary examinations were made prior to the administration of arsphenamin, and were followed up for two successive days after its injection. Thirty cases received neo-arsphenamin and twenty cases the old arsphenamin. It was found, on the whole, that the administration of arsphenamin had no effect on the increase of urobilogen. Only those cases which had somewhat high figures before the administration of arsphenamin gave slightly higher values after its administration. Thirty-nine cases had normal urobilogen figures, and these were not changed after the arsphenamin. In eleven out of the fifty, urobilogen readings before the injections were from 1 : 30 to 1 : 50. Three gave 1 : 30, two 1 : 40, three 1 : 50, one 1 : 75 and two 1 : 150. In one of the cases with secondary reseola in which the urobilogen was up to 1 : 150 before the administra-

43. Heyd, C. G.: The Liver and Its Relation to Chronic Abdominal Infections, Ann. Surg. **79**:55 (Jan.) 1924.

tion of arsphenamin, the urobilogen rose to 1 : 500 the day following its administration; however, it came down to normal within the next few days. Two cases with 1 : 30 urobilogen came up after the old arsphenamin, one to 1 : 75, and one to 1 : 100. One case of 1 : 50 rose to 1 : 125 after old arsphenamin. The remainder of those that gave originally higher readings remained unchanged.

In this connection, some experiments also were made with intravenous injections of neo-arsphenamin in the dog. An injection of 0.45 gm. of this drug produced a rise in urobilogen of 1 : 30 the following day, which fell to normal on the third day. A week later, the injection of 0.9 gm. of neo-arsphenamin produced no rise. The effects of the drug, therefore, on a normal liver appear to be very slight or absent altogether.

*Comparisons Between the Phenoltetrachlorphthalein and Urobilogen Tests in Various Liver Diseases.*—Six cases were selected for comparative study of the phenoltetrachlorphthalein and urobilogen output. They comprised: three cases of catarrhal jaundice, one case of splenomegalia with anemia, one case of syphilis with a large liver, and one case of marked cardiac decompensation with large liver and intense jaundice.

Two cases of catarrhal jaundice gave negative reactions to the phenoltetrachlorphthalein test at the end of one hour. The urobilogen tests on the same day were 1 : 60 and 1 : 70, respectively. The first patient experienced a chill followed, for the next three days, by an increase in the output of urobilogen up to 1 : 200. The third patient with catarrhal jaundice developed a severe pain in the hepatic region promptly following the injection of the dye. He also developed a phlebitis. No reading was taken on this patient.

The case with the cardiac decompensation, which gave constant high urobilogen readings varying from 1 : 300 to 1 : 1,500 gave less than a 1 per cent. phenoltetrachlorphthalein reading.

The splenomegalia case with anemia gave low urobilogen readings up to 1 : 40, and the phenoltetrachlorphthalein was 15 per cent.

The case of syphilis gave persistently low urobilogen readings, whereas the phenoltetrachlorphthalein was 12 per cent.

In the foregoing, we note differences in the behavior of the liver to phenoltetrachlorphthalein and urobilogen. The retention of the dye does not necessarily indicate a deficiency in the performance of the normal metabolic function, but merely points to a lessened capacity of the liver to eliminate foreign substances. It has already been pointed out that, in chronic liver diseases without parenchymatous changes, there occurs a gradual compensation of the liver to perform its normal

metabolic functions. When, however, foreign substances are suddenly thrown into the liver substances to which the liver is not accustomed, then the cell fails, and its eliminative power is retarded.

That the phenoltetrachlorphthalein is not entirely an innocuous substance can be seen from the cases herein reported, in which, in one instance, a severe pain in the hepatic region followed the injection and also a marked increase in the urobilogen output lasting a number of days. The complication of phlebitis has also been reported by others.

TABLE 3.—*Urobilogen Estimations in Urine in Certain Conditions*

| Clinical Diagnosis | Number of Cases | Urobilogen | Bilirubin in Serum |
|---|---|---|---|
| Catarrhal jaundice | 17 | 1:75 to 1:250 | 1:85,000<br>1:67,000<br>1:37,000<br>1:15,000<br>1:10,000 |
| Cholangeitis | 1 | 1:500 | ..... |
| Cholangeitis followed by liver abscess | 1 | 1:200 | 1:35,000 |
| Chronic cholelithiasis with diabetes; subicteric | 1 | 1:50 | 1:110,000 |
| Primary carcinoma of liver (necropsy) | 1 | 1:350 | 1:200,000 |
| Carcinoma of stomach with liver metastasis | 2 | 1:150 to 1:300 | ..... |
| Carcinoma of head of pancreas | 4 | Absent | 1:10,000<br>1:5,000 |
| Carcinoma of stomach | 8 | Normal | ..... |
| Carcinoma of esophagus | 3 | Normal | ..... |
| Carcinoma of ovary and peritoneum | 2 | Normal | ..... |
| Carcinoma of rectum | 2 | Normal | ..... |
| Carcinoma of colon | 1 | Normal | ..... |
| Carcinoma of lung | 2 | Normal | ..... |
| Enlarged liver associated with chronic endocarditis | 10 | 1:30 to 1:150 | 1:200,000 |
| Marked cardiac decompensation associated with intense jaundice (death) | 1 | 1:1,500 | ..... |
| Gastric ulcers | 7 | 2, 1:50; 5, normal | ..... |
| Diabetes, during stage of acidosis | 3 | 1:50 to 1:150 | ..... |
| Cholelithiasis and cholecystitis | 150 | 19, 1:35 to 1:50; 1, acute, 1:100; the rest were normal | 1:48,000 seven cases examined varied from 1:400,000 to 1:200,000 |
| Chronic appendicitis | 65 | 4, 1:35; 61, normal | 1:400,000 to 1:200,000 |
| Hydrops of the gallbladder | 4 | Normal | 1:500,000 to 1:250,000 |
| Tuberculous meningitis | 1 | 1:80 | ..... |

In Table 3 are given the urobilogen estimations in the urine in some of the conditions studied.

The remainder, comprising a series of close to a thousand cases of miscellaneous conditions, such as visceroptosis, nutritional disturbances, chronic infections of the tonsils and teeth, sinusitis, rheumatism, articular involvement, lumbago, psychoneurosis and bronchitis, all gave normal findings.

### (2) HEMOLYTIC DISEASES

*Pernicious Anemia.*—Five cases are here reported, three in the active stage, and two in the terminal or aplastic stage. The first three presented high urobilogen outputs of 1:80, 1:75 and 1:50, respectively. The other two gave low urobilogen readings of 1:20 and 1:10.

The presence of an increased urobilogen in the anemias differentiates the primary from the secondary form. In the former, the excess

urobilogen is derived from the increase in the red blood cell production and destruction which is continuously going on, while, in the latter, there is no increase in the production of the red cells, but a conservation in their destruction, with the result of very low urobilogen elimination, often less than 1 : 10.

The increase of urobilogen in pernicious anemia associated with an icteric tint of the sclera and skin and often a palpable liver presents difficulty at times in the differentiation between hepatic disease and primary anemia. Aside from the study of the morphology of the blood,

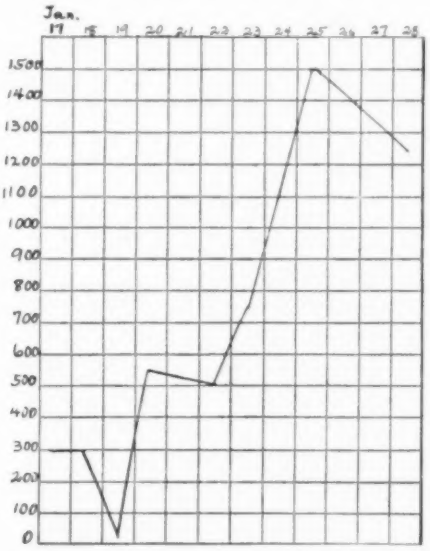


Chart 6.—Urobilogen elimination in case in which there was marked cardiac decompensation, large liver and intense icterus.

the examination of the feces indicates an increased pigmentation to a dark brown color, in counterdistinction to the light or acholic feces accompanying liver disease. In the anemias, bile is never found in the urine, while in hepatic diseases associated with icterus, bile is always present.

*Malaria.*—The increase of urobilogen in malaria is a constant finding. This forms an important factor in the differential diagnosis between malaria and other infectious diseases. The diagnosis of malaria can often be made before the discovery of the parasites in the blood. A routine examination of the urine should always be made in infectious diseases with prolonged high temperatures.

Three cases of tertian malaria from the wards of Bellevue Hospital are here reported. One had the first attack in August, 1923. He came under observation in the hospital, December 11, with a blood count of 2,000,000 red cells, 50 per cent. hemoglobin, and urobilogen, 1:50. December 14, the patient had paroxysms at 11 a. m., with urobilogen 1:75, and at 4 p. m., 1:100. Quinin administration was begun at 4 p. m. The following day, at noon, the urobilogen fell to 1:20, and remained normal after the quinin therapy.

Two other cases of malaria showed, on the day of the paroxysm, 1:300 urobilogen in one, and 1:150 in the other. Both of these cases presented difficulties in the clinical diagnosis. The discovery of a high urobilogen, with febrile paroxysms helped in the diagnosis of malaria prior to the finding of the parasites. The administration of quinin promptly reduced the urobilogen to normal.

## SUMMARY AND CONCLUSIONS

1. A simple quantitative method for urobilogen estimation is described based on the Ehrlich's aldehyd reaction, and consisting of a series of dilutions which are made until no further reaction takes place. It has been found six times as sensitive as the spectroscopic method of Wilbur and Addis.

2. A series of animal experiments are here reported which were undertaken with the objects (1) to prove the relation between liver damage and increase of urobilogen in the urine, and (2) to study the rôle of the intestine in the formation of urobilogen.

The results of these experiments indicate: (*a*) that, in parenchymatous changes of the liver, such as induced by chloroform, there occurs a marked increase in the urinary urobilogen; (*b*) that urobilogen is formed normally in the intestinal canal by a process of decomposition of the bile pigment. In exclusion of bile from the intestinal canal by ligation and excision of the common duct, no urobilogen appears in the urine, even though the liver has undergone marked parenchymatous changes by subsequent chloroform administration. When, however, the flow of bile is reestablished by anastomosis between the gallbladder and duodenum, or when bile is administered by mouth, urobilogen again makes its appearance.

Only in rare instances of intrahepatic infections, as in cholangeitis, may the urobilogen be formed within the biliary radicles. The presence of urobilogen in the liver bile is thus explained by intrahepatic infections and also by excessive amounts of urobilogen being brought to the liver from the intestinal canal.

The finding of small quantities of urobilogen in the intestinal canal in common duct occlusion is due to the excretion of bilirubin from the

general circulation into the intestines and its being there changed to urobilogen.

3. The clinical significance of urobilogen has been studied in a series of over 1,200 cases, comprising catarrhal icterus; cholangeitis; hepatic cirrhosis; cholecystitis and cholelithiasis; carcinomas of abdominal organs; cardiac decompensation; diseases of the hemopoietic system; granulomas, infectious diseases; malaria, and also a group of miscellaneous conditions, such as gastro-intestinal and nutritional disturbances and chronic infections, and including a large series of normal persons.

We find that in the normal urine the urobilogen seldom rises above a 1:20 dilution. In constipation with putrefactive changes, it may become increased somewhat temporarily.

All pathologic conditions in which urobilogen is eliminated in excessive amounts can be classified into two main groups: (1) diseases of the liver and biliary tract, and (2) hemolytic diseases.

In the former, it is associated with parenchymatous changes in the liver, causing an absolute insufficiency in the mobilization of urobilogen, while, in the latter, the increase of urobilogen is due to an increase in the bile pigment formation which produces a relative insufficiency of the liver. Both conditions may at times occur simultaneously, and, in fact, it seems quite probable that some liver damage also is present in hemolytic diseases.

Urobilogen is found in largest amounts in the acute and subacute liver changes and in exacerbations in the course of chronic processes. In the slowly progressive chronic liver lesion, the output of large amounts of urobilogen is not constant. This is probably due to the ability of the organ to perform its normal metabolic function by compensatory growth of new liver tissue.

In comparing the phenoltetrachlorphthalein to the urobilogen test in various liver affections, the different results may be explained by the difference in behavior of the liver cell toward foreign substances. Their retention does not always signify an insufficient metabolic liver function.

4. The constant presence of urobilogen in catarrhal jaundice, and its total absence in mechanical icterus, such as encountered in carcinoma of the head of the pancreas and of the biliary tract, forms a marked distinguishing diagnostic feature between this benign form of jaundice and those due to malignant causes.

5. Finally, we wish to advocate the use of the urobilogen test as a routine laboratory measure in all urine examinations. In icterus or latent icterus, in hepatic disorders, in the anemias, in malaria, in infections, in malignancies and in intoxications, it will supply an important adjuvant in the routine diagnosis, and will often aid in clearing up intricate and obscure clinical conditions.

# BLOOD VOLUME

## IV. DIURNAL FLUCTUATIONS IN BLOOD VOLUME AND CHANGE INCIDENT TO TRANSFUSION REACTION *

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Diurnal fluctuations in the count of corpuscles derived from a transfusion appear to have a consistent trend in patients with anemia, and are assumed to indicate blood volume changes. Changes incident to transfusion, pointing to a reduced blood volume following a transfusion reaction, have also been noted.

Little attention has been given to rapid fluctuations in the blood count, and the possibility of their occurrence is often lost sight of. Limbeck [1] studied the changes in the red and white cell count after eating, and reports a drop in the red cell count. He found a count of 5,550,000 before the meal, 5,320,000 a quarter of an hour after the meal, 5,480,000 an hour and a quarter after, 4,730,000 two hours and a quarter after, 4,870,000 three and a quarter hours after, and 4,720,000 four hours and a quarter after. In reviewing the literature, he found that Sorenson noted an increase in the red cells of from 15 to 19 per cent. about an hour after food was taken, while Reinhardt, Vierdt and Duperi found a diminution. Dreyer [2] has recently studied the question of diurnal changes in the hemoglobin, making observations at short intervals throughout the twenty-four hours by means of a technic which he considers accurate to within a few per cent. He gives five representative curves. In one of these there is a definitely progressive drop in the hemoglobin percentage from 10 a. m. to 4 p. m., with a subsequent rise, reaching its peak at 4 a. m. In the other curves are marked irregularities that seem to have no definite trend. Dreyer considers that the fluctuations are irregular, but that the late afternoon is a time at which the hemoglobin percentage readings are more stable, and that the greater fluctuations occur in patients with anemia. Dreyer obtained the blood for the determinations from a freely dripping puncture. Rabinovitch [3] more recently studied the variation of the hemoglobin percentage in twenty normal subjects, taking readings every two hours from 8 a. m. to 6 p. m.

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* From the Mayo Foundation.

1. Limbeck, R. R.: The Clinical Pathology of the Blood, London, New Sydenham Society, 1901, p. 184.

2. Dreyer, G.; Bazett, H. C., and Pierce, H. F.: Diurnal Variations in the Hemoglobin Content of the Blood, Lancet **2**:588-591 (Sept. 18) 1920.

3. Rabinovitch, I. M.: Variations of the Percentage of Hemoglobin in Man During the Day, J. Lab. & Clin. Med. **9**:120-123 (Nov.) 1923.

He noted maximal variations ranging from 1.1 to 26.2 per cent. with no definite trend to the variations. It is perhaps a matter of coincidence; but, as his figures are given, it happens that in the four subjects showing the greatest maximal variation, 20.2, 21.4, 26 and 26.2 per cent., the low percentages occur in the afternoon.

It would seem more probable that these changes in the hemoglobin were either due to redistribution of the corpuscles, or to changes in plasma volume, and not to destruction and production of corpuscles. The latter possibility I have, in part at least, ruled out by testing the fluctuations in the counts of the transfused corpuscles, and finding that they, too, show fluctuations which tend to parallel those found in the native red blood count, the discrepancies between the two sets of readings being inconsiderable, in view of the degree of accuracy of the technic, although it is conceivable that there might be some significance to them.

My interest in diurnal fluctuations in the cell count as a probable indication of blood volume change was enlisted by the marked differences that were often noted between counts taken at different times of the day in patients with pernicious anemia, who were at the beginning of a course of treatment. As the patient improved, there was a tendency for the counts to become less irregular. One case (Case 80) especially was studied from this point of view. Counts were taken between 8 and 9 a. m., and in the evening. The study was begun immediately after the first transfusion. The fluctuations of the count of unagglutinable corpuscles indicated at first volumes which changed from 72 to 47 c.c. for each kilogram of body weight, the higher volume being found in the morning. In the course of nine days, as the patient's condition improved with rest in bed and forced fluids, the volume, as determined by the count of unagglutinable corpuscles, gradually increased to 83 c.c. for each kilogram of body weight, and the fluctuations became negligible. This increase was not accompanied by any marked improvement in the number of native corpuscles found in each cubic millimeter of blood, although if the interpretation of the decrease in the number of unagglutinable corpuscles, as indicating a blood volume increase, is correct, the total number of corpuscles in the circulation was considerably augmented. As compared with the fluctuations reported for normal subjects by Rabinovitch, and for normal and anemic subjects by Dreyer, the fluctuations, in this subject, were greater at the beginning of treatment, and less after improvement had taken place.

Studies were made of blood volume changes through the day. It was not practicable to make many determinations in one day, but four consecutive studies were made on a patient with pernicious anemia, who was in bed in the hospital; the count remained low throughout the study.

Two studies were made on a patient with pernicious anemia, who was out of the hospital and in better condition, and two more intensive studies were made on a patient with purpura, who was fairly well. Also a small

TABLE 1.—*Diurnal Variation in Blood Red Cell Count Unagglutinable Corpuscles*

| | | | Red Cell Count | | Unagglutinable Corpuscles | | |
|---|---|---|---|---|---|---|---|
| Case | Date | Time | In Millions | Per Cent. Change | In Millions | Per Cent. Change | Diagnosis and Remarks |
| 151 | 3/10/21 | Transfusion with unlike group blood | | | | | |
| | 3/11/21 | 9:00 a.m. | 2.12 | 0 | 0.760 | 0 | Pernicious anemia |
| | | 4:15 p.m. | 1.53 | −28 | 0.628 | −16 | |
| | 3/14/21 | 9:15 a.m. | 1.64 | 0 | 0.578 | 0 | |
| | | 3:00 p.m. | 1.39 | −15 | 0.529 | − 8 | |
| | | 5:00 p.m. | 1.28 | − 5 | 0.556 | − 4 | |
| | 3/16/21 | 9:00 a.m. | 1.88 | 0 | 0.701 | 0 | From ear |
| | | 9:00 a.m. | 1.61 | 0 | 0.649 | 0 | From vein |
| | | 12:00 m. | 1.59 | −15 | 0.657 | − 6 | From ear |
| | | 3:30 p.m. | 1.57 | −15 | 0.583 | −17 | From ear |
| | | 3:30 p.m. | 1.10 | −28 | 0.498 | −22 | From vein |
| | | 7:00 p.m. | 1.75 | −13 | 0.680 | − 3 | From ear |
| | 3/17/21 | Transfusion | | | | | |
| | 3/24/21 | 9:10 a.m. | 1.94 | 0 | 0.594 | 0 | |
| | | 11:30 a.m. | 1.94 | 0 | 0.573 | − 4 | |
| | | 3:20 p.m. | 2.07 | + 6 | 0.524 | −12 | |
| | | 9:30 a.m. | 1.91 | − 1 | 0.602 | + 1 | |
| 97 | 9/ 3/20 | 10:00 a.m. | 2.70 | 0 | 0.534 | 0 | From ear. Pernicious anemia |
| | | | | | 0.529 | 0 | From vein |
| | | 2:30 p.m. | 2.65 | − 5 | 0.641 | +20 | From ear |
| | | | | | 0.583 | +10 | From vein |
| | | 9:00 a.m. | 2.09 | −23 | 0.513 | − 4 | From ear |
| | | | | | 0.584 | +10 | From vein |
| | 9/10/20 | 9:30 a.m. | 2.82 | 0 | 0.683 | 0 | From ear |
| | | | | | 0.622 | 0 | From vein |
| | | 3:00 p.m. | 2.45 | −13 | 0.657 | − 4 | From ear |
| | | | | | 0.513 | −16 | From vein |
| | | 8:15 a.m. | 2.47 | −12 | 0.590 | −14 | From ear |
| | | | | | 0.576 | − 7 | From vein |
| 116 | 2/ 6/20 | 7:45 a.m. | .... | .... | 0.490 | − 4 | In bed. Purpura |
| | | 9:45 a.m. | .... | .... | 0.502 | − 2 | In bed |
| | | 11:00 a.m. | Breakfast | .... | .... | .... | Up and about |
| | | 11:30 a.m. | .... | .... | 0.511 | 0 | |
| | | 1:15 p.m. | 5.04 | .... | 0.512 | 0 | |
| | | 1:30 p.m. | Luncheon | | | | |
| | | 3:00 p.m. | .... | .... | 0.499 | − 4 | |
| | | 5:30 p.m. | .... | .... | 0.446 | −13 | |
| | | 6:00 p.m. | Dinner | | | | |
| | | 6:40 p.m. | 5.14 | .... | 0.466 | − 9 | |
| | | 9:50 p.m. | .... | .... | 0.533 | + 8 | |
| | | 12:40 a.m. | 5.33 | .... | 0.520 | + 2 | |
| | 2/11/24 | 8:25 a.m. | 5.04 | − 7 | 0.423 | −13 | |
| | | 10:15 a.m. | Breakfast | | | | |
| | | 10:55 a.m. | 5.42 | 0 | 0.488 | 0 | |
| | | 11:20 a.m. | .... | .... | 0.486 | 0 | |
| | | 11:40 a.m. | 5.25 | − 4 | 0.487 | 0 | |
| | | 12:10 p.m. | .... | .... | 0.470 | − 4 | |
| | | 1:15 p.m. | Luncheon | | | | |
| | | 1:30 p.m. | 5.00 | − 8 | 0.435 | −11 | |
| | | 2:55 p.m. | .... | .... | 0.438 | −10 | |
| | | 3:55 p.m. | .... | .... | 0.442 | − 9 | |
| | | 5:15 p.m. | 5.17 | .... | 0.453 | − 7 | |
| | | 6:45 p.m. | Dinner | | | | |
| | | 7:30 p.m. | 5.38 | .... | 0.500 | +12 | |
| | | 9:00 p.m. | Hot lemonade | | | | |
| | | 9:15 p.m. | .... | .... | 0.413 | −13 | |
| | | 11:15 p.m. | .... | .... | 0.430 | −12 | |
| | | 12:50 p.m. | .... | .... | 0.438 | −10 | |
| 80 | 8/31/19 | 10:00 a.m. | 1.93 | 0 | 0.522 | 0 | Pernicious anemia |
| | | 3:00 p.m. | 1.99 | + 3 | 0.545 | + 4 | |
| | | 6:00 p.m. | 2.06 | + 6 | 0.564 | + 8 | |

study on a patient previously mentioned (Case 80) is included because it proved an exception to the trend in the change of count. In some instances, counts were made both on blood from the ear and on blood

from venipuncture. The percentage of change is based on the first count obtained after breakfast. Duplicate or triplicate counts were made in all instances, and only those which gave satisfactory checks are included in Table 1.

In the case of the patient with pernicious anemia who was in bed in the hospital, and who was tested four times, there was a lower count, indicating a greater blood volume in the afternoon than in the morning

TABLE 2.—*Variation in Blood Volume as Indicated by Variations in the Cell Count Following Transfusion*

| Case | | Time After Transfusion | Red Cell Count in Millions | Unagglutinable Corpuscles in Millions | Blood Volume | Temperature | Remarks |
|---|---|---|---|---|---|---|---|
| 97 | Before | ..... | 1.99 | 0.070 | ..... | ..... | |
| | | 10 minutes | 1.89 | 0.496 | 5,350 | ..... | |
| | | 35 minutes | 2.20 | 0.498 | 5,350 | 99.2 | Chill beginning |
| | | 39 minutes | .... | 0.543 | 4,800 | ..... | Severe shivering |
| | | 42 minutes | .... | 0.566 | 4,600 | ..... | Severe shivering |
| | | 70 minutes | 1.78 | 0.495 | 5,350 | 102.2 | |
| | | 4 hours | 2.24 | ..... | ..... | 102.4 | |
| | | 18 hours | 2.19 | 0.405 | 6,100 | ..... | |
| 94 | Before | ..... | 2.09 | 0.440 | 4,900 | 98.7 | Patient in Group 4 received a Group 4 transfusion following a Group 2 transfusion. Unagglutinated corpuscles are Group 2 corpuscles |
| | | 40 minutes | 2.60 | 0.580 | 3,700 | 100.8 | Slight chill |
| | | 3 hours | 2.58 | 0.553 | 3,900 | ..... | |
| | | 18 hours | 2.25 | 0.448 | 4,700 | ..... | |
| 212 | Before | ..... | 1.16 | 0.031 | ..... | ..... | |
| | | 10 minutes | 1.10 | 0.646 | 3,820 | ..... | Vomited |
| | | 40 minutes | 1.61 | 0.724 | 3,400 | 102 | |
| | | 18 hours | 1.64 | 0.737 | 3,340 | 99.2 | |
| 207 | Before | ..... | 1.75 | ..... | ..... | ..... | |
| | | 10 minutes | 2.14 | ..... | ..... | ..... | Lymphatic leukemia, high count of white cells, no transfusion reaction |
| | | 40 minutes | 2.08 | ..... | ..... | ..... | |
| | | 70 minutes | 2.13 | ..... | ..... | ..... | |
| | | 18 hours | 2.07 | ..... | ..... | ..... | |
| 116 | Before | ..... | 4.58 | ..... | ..... | ..... | |
| | | 10 minutes | 4.79 | 0.556 | ..... | ..... | No transfusion reaction |
| | | 40 minutes | 4.82 | 0.566 | ..... | ..... | |
| | | 18 hours | 4.96 | ..... | ..... | ..... | |
| 29 | Before | ..... | 2.06 | 0.128 | ..... | ..... | Group 1 transfusion |
| | After | ..... | 2.56 | 0.687 | 3,400 | ..... | |
| | | 18 hours | 3.06 | 0.760 | ..... | ..... | Chill day after transfusion |
| | | 2 days | 2.23 | 0.548 | 4,600 | ..... | |

or evening. The counts on the patient with purpura, who was ambulatory, indicated that the blood volume was high while he was in bed in the morning, became less after he had been up and had had his breakfast, increased further in the afternoon, and decreased after dinner on both days that the tests were run, although on one day, when hot lemonade was taken at 9 in the evening, there was an increase in volume which was still evident at midnight. In the case of the patient with pernicious anemia, who was in the better condition, there was an increase in the blood volume in the afternoon on one day, but on the occasion of the other test, there were irregularities in the results for which I cannot

account. These data would, on the whole, indicate that there is a tendency for the blood volume to increase in the afternoon in some persons who are not normal. On the other hand, in six comparisons between the morning and afternoon counts which were made on a normal subject, no definite tendency appeared. The maximal percentage of variation was considerably less.

Of interest in this connection is some work reported by Price-Jones [4] on the diurnal variation in the size of the red corpuscles. He finds considerable variation due to changes in the hydrogen ion concentration of the blood. In the morning before any exercise has been taken, he finds the corpuscles smallest. They increase in size during the day and fall again after midnight. The investigation was made on his own blood. Of the three curves that were made, one was representative of a study of the changes which occurred while the subject remained in bed for twenty-four hours. In this curve, although the general shape is the same as that of the other two taken during a normal day's activity, there is a marked fall in the size of the average diameter of the corpuscles between 3 and 6 p. m., during which time two observations were made. Although the author does not stress this point, it is of interest in view of the change in blood volume found in patients during this time, as the results obtained from the subject kept in a state of comparative inactivity would be likely to be more comparable to those obtained from a patient whose activity is reduced.

Changes in weight in tuberculous patients, reported by Scott,[5] are also if interest in this connection. The patients were weighed at 10 a. m., and between 2 and 4 p. m. Of 962 patients, 611 weighed more in the afternoon than in the morning, the difference being from 0.25 to 4 pounds. In the positive cases, there was a greater difference between the morning and afternoon weights than in the suspects, and the more advanced the disease, the greater the difference. As the patients who were in the worse condition had the greatest increase in weight in the afternoon, the figures would suggest that there was some factor other than the increase in weight of the intestinal contents resulting from the intake of food, and that the body tissues themselves, of which the blood may have been the most important, increased their water content.

Changes in hydrogen ion concentration of the blood immediately suggest themselves as the cause of the changes in blood volume, but no parallel studies of hydrogen ion concentration of the blood were made. Changes in hydrogen ion concentration would be expected to affect the

4. Price-Jones, C.: The Diurnal Variation in the Sizes of Red Blood Cells, J. Path & Bacteriol. **23**:351-383 (Dec.) 1920.

5. Scott, J. M.: Diurnal Variation in Body Weight in Tuberculous Patients, Lancet **2**:639-640, 1919.

plasma volume and the total corpuscle volume in opposite directions. We know that an increase in hydrogen ion concentration within the range of the hydrogen ion concentration of the blood causes a decrease in corpuscle volume. Although I have seen no work on the variation in osmotic pressure of blood serum with variation in hydrogen ion concentration, I think it safe to assume, in view of the work of Sorenson [6] and Loeb [7] on egg albumin and gelatin, that both the chief constituents of the blood serum increase in osmotic pressure with an increase in alkalinity within the range of the hydrogen ion concentration of the blood, although in view of the fact that their iso-electric points are different, the curves for their rate of change within the same range of hydrogen ion concentration may be quite unlike. The change in the whole blood volume would be due to the combined effect of the decrease in the size of the corpuscles and of the increase in osmotic pressure of the serum proteins. The sum of these changes, if this is the explanation, is apparently smallest in normal subjects in whom the corpuscle and plasma volumes are more nearly equal, and greater in anemic subjects in whom the corpuscle volume is diminished. On this basis, it would seem that the increase in the plasma volume usually approximately balances the decrease in corpuscle volume caused by decrease in hydrogen ion concentration of the blood in normal subjects, whereas in anemic subjects the corpuscle decrease in volume is not enough to balance the serum protein increase in osmotic pressure producing the more unstable blood volume. It would seem probable also that differences in the proportion of serum albumin and serum globulin may play a part. A study of the tendency to instability of blood volume in correlation with the protein composition of the blood would be of interest. The fact that this increase in blood volume in the afternoon has tended to appear in patients who are inactive, and not in the normal subject studied, may also be due to some physiologic alkaline tide, perhaps that of digestion, which in the normal subject was masked by bodily activity.

### CHANGE OF VOLUME DURING TRANSFUSION REACTIONS

A few studies were made on patients following transfusion, both during a reaction and when there was no reaction. As the transfusions were given in the early afternoon, and reactions usually occur in one-half to one and one-half hours after transfusion, the time of day at which these transfusion reactions occurred was that at which normally there would tend to be an increase in blood volume. My few studies

6. Sorenson, S. T.: Studies on Proteins, Compt. rend. Lab. de Carlsberg, Copenhagen, 1917.

7. Loeb, J.: Proteins and the Theory of Colloidal Behavior, Ed. 1, New York, McGraw-Hill Book Company, 1922.

would seem to indicate that whether or not there was an initial increase in blood volume following transfusion, over and above that produced by the transfusion itself, when a transfusion reaction occurred, it was accompanied by a decrease in volume. This decrease was not observed in cases in which no reaction resulted from transfusion. From the data given in Table 2, it will be seen that the magnitude of the decrease in volume is of about the same order as the greater increase in volume found in the more sensitive patients in the diurnal change at this time.

The counts from which these volume computations were derived were made on blood from an ear puncture, and may have been influenced by the admixture of blood from the capillaries, in which a greater tendency to congestion is possible on puncture during the transfusion reaction than before. As simultaneous venipuncture was not done, this possibility cannot be ruled out. I am, however, of the opinion that it is the less probable explanation of the increased count, and that this was more probably due to a decrease in blood volume. The greater instability of blood volume, which appears to exist in patients who are in the initial stages of their treatment, and the finding of Butsch and Ashby,[8] that in a series of 265 transfusions, eighty-one patients with a hemoglobin below 30 per cent. had a markedly greater tendency to reaction (45 per cent. against 35 per cent.), would make it seem probable that there may be some common ground for the tendency to instability of blood volume and the tendency to transfusion reaction, since these patients with the lower hemoglobin were on the whole those who were in the initial stages of their treatment.

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8. Butsch, J. L., and Ashby, Winifred: The Effect of the Digestive Period and Other Factors in Reactions After Blood Transfusions, New York M. J. **113**:513-517 (April 6) 1921.

# BLOOD VOLUME

## V. THE EFFECT OF TREATMENT ON THE BLOOD VOLUME OF PATIENTS WITH PERNICIOUS ANEMIA *

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The blood volume of patients with pernicious anemia was calculated from the count of unagglutinable corpuscles during the course of a series of transfusions. The number of corpuscles for each cubic millimeter was counted, the total number of corpuscles present in the circulation computed, and an attempt was made to correlate the three factors, blood volume, red cell count and total number of corpuscles, with the general condition of the patient.

The studies were started simultaneously with the treatment of a patient who either had not been treated before, or who had come back for a course of treatment after a relapse. A transfusion of unagglutinable corpuscles was given first, followed by a series of transfusions of blood of the same group as that of the patient. These studies were made incidental to a study of the rate of elimination of transfused blood in patients with pernicious anemia. At the time when they were made, it was not feasible to confirm by other methods the changes in volume which apparently occurred, although, in some instances, it was possible to make a partial check by means of a second unlike group transfusion. A blood volume, however, determined from change in unagglutinable corpuscles before and after transfusion, is open to the possibility of error already considered;[1] but, as the number of corpuscles present before transfusion is not greater than the number added, the error which might be introduced by fluctuations in blood volume would be such that the result may be regarded as of value for verification. The results have also been checked to a certain degree by a comparison of the maximal blood volumes in this series with those in similar cases by other workers using the vital red method. The possibility that the apparent increase in the blood volume resulting in many instances from a series of transfusions might be due to destruction of the unagglutinable corpuscles initially transfused, rather than to their dilution, was still further investigated, in some cases, by giving the transfusion of unagglutinable blood from which the blood volume was derived as a later member in a series of transfusions. It was then possible to compare the

* From the Mayo Foundation.

1. Ashby, Winifred: Blood Volume. III. Apparent Changes in Blood Volume Indicated by Transfusion, and Their Bearing on Methods of Determining Blood Volume by Means of the Degree of Change in a Constituent of the Blood, Following Transfusion of a Known Amount of That Constituent, Arch. Int. Med. **35**:641 (May 15) 1925.

blood volume so obtained with volumes indicated after a similar degree of treatment in cases in which the transfusion of unagglutinable blood was the initial transfusion.

The patient's own impression of his condition, my impressions as I saw him in the course of the experiment, the hospital records, and the clinician's résumé of the case, before and after treatment, were made use of in determining the change in condition due to treatment. As Lindeman [2] considered that there was a correlation between a high blood volume and the ability to endure a low cell count without so much inconvenience from "palpitation of the heart and ringing in the ears," the patients were questioned as to this point. Note was made of throbbing sensations in the body and ringing in the ears.

Of the numerous cases studied, the complete data on a few which seem to be representative are given, and also the data on one (Case 59), which seems to be exceptional. The patients may be classified as follows: (1) those whose blood volume and cell count were initially low, who were in very bad condition, and who improved without any considerable increase in cell count as determined by the cell content for each cubic centimeter of blood, but with a comparatively marked increase in blood volume and total cell count; (2) those who improved only little, and whose apparent blood volume and cell count remained low; (3) those who had a nearly normal blood volume, and were in fairly good condition before treatment, and who made a good recovery following transfusion, and (4) an occasional case in which the initial blood volume and count were low, and in which there was an improvement in the cell count before there was an increase in blood volume.

## REPORT OF CASES

CASE 70.—A man, aged 62, had pernicious anemia. He was in very poor condition. On physical examination, a pulsating wave in the upper abdomen was noted (probably pulsating aorta), and a bruit over the femoral vessels. He improved markedly under treatment, and became able to take fairly long walks.

TABLE 1.—*Findings in Case 70*

| Days of Treatment | Red Cell Count in Millions | Corpuscles to Each Kilogram of Body Weight | C.c. of Blood for Each Kilogram of Body Weight |
|---|---|---|---|
| Transfusion of unlike group blood | | | |
| 0 | 1.02 | $55 \times 10^9$ | 54 |
| 1 | 1.28 | $96 \times 10^9$ | 75 |
| 3 | 1.13 | $89 \times 10^9$ | 79 |
| 4 | 0.88 | $77 \times 10^9$ | 88 |
| 4 Transfusion | | | |
| 5 | 1.11 | $108 \times 10^9$ | 97 |
| 8 | 1.40 | $131 \times 10^9$ | 94 |
| 11 Transfusion | | | |
| 14 | 1.79 | $159 \times 10^9$ | 89 |
| 17 | 1.62 | $167 \times 10^9$ | 103 |
| 18 | 1.64 | $177 \times 10^9$ | 120 |

2. Lindeman, E.: The Total Blood Volume in Pernicious Anemia, J. A. M. A. **70**:1292-1297 (May 4) 1918.

CASE 43.—A man, aged 49, had pernicious anemia. He was given the first series of transfusions and died three years later.

TABLE 2.—*Findings in Case 43*

| Days of Treatment | Red Cell Count in Millions | Corpuscles to Each Kilogram of Body Weight | C.c. of Blood for Each Kilogram of Body Weight | Remarks |
|---|---|---|---|---|
| | Transfusion of unlike group blood | | | |
| 1 | 1.95 | $119 \times 10^9$ | 61 | Ringing in the ears and throbbing |
| 2 | 1.86 | $124 \times 10^9$ | 67 | |
| 4 | 1.71 | $152 \times 10^9$ | 89 | Ringing and throbbing stopped |
| 8 | Transfusion | | | |
| 11 | 2.20 | $194 \times 10^9$ | 88 | |
| 14 | 1.99 | $154 \times 10^9$ | 83 | |
| 14 | Transfusion | | | |
| 14 | 2.40 | $230 \times 10^9$ | 96 | |
| 36 | 2.40 | $258 \times 10^9$ | 108 | |
| 45 | 2.32 | $259 \times 10^9$ | 108 | Patient feels much better |
| 51 | .... | ..... | 85 | Patient weaker |
| 53 | 2.16 | $181 \times 10^9$ | 84 | Patient stronger |
| 56 | 1.84 | $157 \times 10^9$ | 85 | Patient stronger |
| 59 | Drop occurred in the count of transfused corpuscles indicating elimination; ringing in ears and throbbing commenced again | | | |
| 60 | Transfusion | | | |
| 66 | 2.13 | ..... | .. | Ringing in ears stopped |
| 66 | Transfusion | | | |
| 68 | 2.16 | ..... | .. | |
| 91 | Transfusion of unlike group blood | | | |
| 91 | 2.39 | $201 \times 10^9$ | 84 | |

CASE 57.—A man, aged 63, had pernicious anemia. The patient complained of ringing in his ears, and a throbbing sensation. He had noticed weakness for the last two years. On examination just before transfusion, a soft, booming systolic murmur and edema were noted. The patient received the first series of transfusions.

TABLE 3.—*Findings in Case 57*

| Days of Treatment | Red Cell Count in Millions | Corpuscles to Each Kilogram of Body Weight | C.c. of Blood for Each Kilogram of Body Weight | Remarks |
|---|---|---|---|---|
| | Transfusion of unlike group blood | | | |
| 0 | 1.54 | $85 \times 10^9$ | 55 | Ringing in ear and throbbing sensation |
| 1 | .... | ..... | 55 | |
| 2 | 1.55 | $79 \times 10^9$ | 51 | Ringing in ear and throbbing sensation |
| 4 | 1.31 | $66 \times 10^9$ | 50 | Ringing in ear and throbbing sensation |
| 6 | 1.65 | $89 \times 10^9$ | 54 | Ringing in ear and throbbing sensation |
| 7 | 1.72 | $88 \times 10^9$ | 51 | Ringing in ear and throbbing sensation |
| 9 | 1.89 | $104 \times 10^9$ | 55 | Ringing in ear and throbbing sensation (felt better; gain in weight) |
| 11 | 1.95 | $113 \times 10^9$ | 58 | Ringing in ear and throbbing sensation |
| 13 | 2.17 | $107 \times 10^9$ | 50 | Ringing in ear and throbbing sensation |
| 14 | 1.53 | $106 \times 10^9$ | 69 | Less ringing and throbbing |
| 14 | Transfusion | | | |
| 15 | 2.24 | $166 \times 10^9$ | 74 | Ringing and throbbing ceased |
| 20 | 1.84 | $127 \times 10^9$ | 69 | |
| 21 | Teeth extracted | | | |
| 25 | 1.87 | $122 \times 10^9$ | 65 | Ringing in ears at night |
| 27 | 1.87 | $127 \times 10^9$ | 68 | |

Two months later, the patient returned, complaining of ringing in the ears and throbbing in the abdomen. He received a like group transfusion, soon after which these symptoms disappeared. An unlike group transfusion, following this, gave the data for a blood volume estimate, which was 111 c.c. for each kilogram of body weight. At this time, the red cell count was 1,480,000, and the total cells for each kilogram of body weight was 164 by 10.° A

second Group 1 transfusion, seven days later, gave data for a volume estimate of 101 c.c. for each kilogram of body weight. After a fourth transfusion, in this series, the patient was dismissed. The clinician's summary was "appetite better, slight weight increase, not stronger, not much improvement."

CASE 54.—A man, aged 44, had pernicious anemia. The patient did not complain of ringing in the ears or throbbing. He received a series of three transfusions. He returned five months later in fair condition, and died seven months later.

TABLE 4.—*Findings in Case 54*

| Days of Treatment | Red Cell Count in Millions | Corpuscles to Each Kilogram of Body Weight | C.c. of Blood for Each Kilogram of Body Weight |
|---|---|---|---|
| Transfusion | | | |
| 0 | .... | ..... | 68 |
| 3 | 1.59 | $106 \times 10^9$ | 67 |
| 6 | 1.63 | $130 \times 10^9$ | 80 |
| 6 Transfusion | | | |
| 7 | 1.84 | $157 \times 10^9$ | 85 |
| 11 | 2.17 | $145 \times 10^9$ | 67 |
| 12 Transfusion | | | |
| 12 | 2.20 | $150 \times 10^9$ | 68 |
| 13 | 2.12 | $158 \times 10^9$ | 72 |
| 17 | 2.05 | $140 \times 10^9$ | 68 |

CASE 59.—An obese woman, aged 52, had pernicious anemia. She had had previous transfusions and a remission of symptoms. At the time of this study her blood count was 1,330,000. She was given an unlike group transfusion of 475 c.c., after which she greatly improved; her health became practically normal, and remained so for about three years. She did not complain of throbbing sensations or ringing in the ears.

TABLE 5.—*Findings in Case 59*

| Days of Treatment | Red Cell Count in Millions | Corpuscles to Each Kilogram of Body Weight | C.c. of Blood for Each Kilogram of Body Weight | Remarks |
|---|---|---|---|---|
| Transfusion of unlike group blood | | | | |
| 0 | 1.88 | $92 \times 10^9$ | 49 | |
| 1 | 1.77 | $74 \times 10^9$ | 42 | |
| 7 | 1.52 | $71 \times 10^9$ | 47 | |
| 11 | 1.61 | $83 \times 10^9$ | 52 | |
| 22 | 2.98 | $90 \times 10^9$ | 31 | Very hot weather; patient felt well |
| 26 | 3.55 | $114 \times 10^9$ Alkaline | 32 | |
| 28 | 3.47 | $115 \times 10^9$ reserve | 33 | |
| 31 | .... | ..... | .. | Began taking sodium bicarbonate, 8 gm. required to make urine alkaline |
| 33 | 3.97 | $306 \times 10^9$ | 77 | |
| 34 | .... | ..... | 68 | |
| .. | .... | ..... | 73 | |
| 38 | 4.02 | $340 \times 10^9$ | 82 | |
| 40 | 4.50 | $396 \times 10^9$ | 88 | |
| 43 | 4.79 | $392 \times 10^9$ | 82 | |
| 57 | 4.89 | $430 \times 10^9$ | 88 | |

CASE 97.—A man, aged 42, had pernicious anemia. He did not complain of ringing in the ears, or of throbbing sensations. He received his first series of transfusions, and became comparatively well. Later, a splenectomy was performed. He was in good health four years after this study.

TABLE 6.—*Findings in Case 97*

| Days of Treatment | Red Cell Count in Millions | Corpuscles to Each Kilogram of Body Weight | C.c. of Blood for Each Kilogram of Body Weight |
|---|---|---|---|
| Transfusion of unlike group blood | | | |
| 0 | 1.89 | $142 \times 10^9$ | 75 |
| 1 | 2.19 | $211 \times 10^9$ | 96 |
| 6 | 2.12 | $242 \times 10^9$ | 114 |
| 10 | 2.12 | $193 \times 10^9$ | 91 |
| 11 | 2.86 | $266 \times 10^9$ | 93 |
| 13 | 2.87 | $250 \times 10^9$ | 87 |
| 15 | 2.75 | $256 \times 10^9$ | 94 |
| 17 | 2.42 | $245 \times 10^9$ | 101 |
| 17 Transfusion | | | |
| 27 | 2.55 | $222 \times 10^9$ | 87 |
| 28 Transfusion | | | |
| 31 | 3.36 | $316 \times 10^9$ | 94 |
| 35 | 3.03 | $240 \times 10^9$ | 79 |
| 42 | 2.87 | $244 \times 10^9$ | 85 |
| 49 Patient home in excellent condition. Ten months later, after a series of transfusions which were ineffective, a splenectomy was performed, followed three days later by a transfusion | | | |
| Transfusion | | | |
| 25 Transfusion of unlike group blood | | | |
| 26 | 2.70 | $175 \times 10^9$ | 65 |
| 31 | 2.60 | $162 \times 10^9$ | 62 |
| 78 | .... | ..... | 80 |
| 78 Transfusion of unlike group blood | | | |
| 78 | 2.44 | $224 \times 10^9$ | 92 |

COMMENT

Case 70 is a striking example of Group 1. In a similar case, the increase in blood volume was verified by a second unlike group transfusion. Case 43 is a less striking illustration of the same group, in that the blood volume (61 c.c. for each kilogram of body weight) and blood count were not so low initially, and the patient was in better condition. In this case, after the almost complete elimination of the unlike group transfusion, a blood volume determination was made possible by a second unlike group transfusion following two like group transfusions. A blood volume of 84 c.c for each kilogram of body weight was found, which was comparable to the blood volume that had apparently previously been established by a series of transfusions. This finding increases the probability that the improvement in blood volume which apparently occurred during the first series of transfusions was a reality, and not the result of an elimination of unlike group transfused corpuscles. Cases 57 and 54 illustrate Group 2. Case 97 illustrates Group 3. A series of transfusions rendered this patient's blood nearly normal for a time. A splenectomy, which was performed after a stubborn relapse, was followed by a poorer condition; the blood volume was lower and took longer to become normal. Case 59 is exceptional in that a single transfusion initiated a marked and prolonged remission. The woman was obese and her blood volume was initially low. It apparently reached the low figure of 31 c.c. for each kilogram of body weight during some very hot weather, without any inconvenience to the patient. However, as the patient's skin tended to be flushed, I am

inclined to suspect that the high count in the unagglutinable corpuscles which caused the calculation of a low blood volume may have been produced to some extent by congestion of corpuscles in the capillaries. Thirty-one days after the transfusion, coincident with the administration of small daily doses of sodium bicarbonate, there was a drop in the unagglutinable corpuscles. This, if due to increase in blood volume, indicates the establishment of a normal blood volume. The cell count, at this time, increased to 4,000,000. At this length of time after transfusion, however, a drop in the number of transfused cells is more safely regarded as their elimination.

The higher values obtained in this series are of the same order as those reported by Keith [3] using the vital red method. They are usually in the vicinity of 85 c.c. for each kilogram of body weight; in a few instances, they are over 90; values of 103, 108 and 114 appear. One value of 120, which appeared at the end of the series in Case 70, may well have been due to destruction of transfused corpuscles, although it is not out of keeping with values reported by Keith in cases of leukemia. In a series of nineteen determinations reported by Keith on patients with pernicious anemia, nine had volumes ranging from 81 to 89, one had a volume of 92, one 102, and one 106 c.c. for each kilogram of body weight. Keith, unlike Bock [4] finds much variation in plasma volume percentages among different patients, and also in individual patients. Although the results given here have not been calculated in terms of plasma volume, it is obvious that the increases indicated would, in most cases, have to be mainly in the plasma volume, as the corpuscle increase was usually not sufficient to account for the difference.

There were a few instances in which the unlike group transfusion, from which the blood volume determination could be made, was not the first transfusion. The immediate blood volume calculations obtained in these cases are parallel to the values indicated at the same length of time after the initial transfusion in cases in which the unagglutinable blood was given at the first transfusion, and had consequently been in the circulation for some little time when the determinations were made. This would still further support the validity of the apparent increases in blood volume on a series of transfusions seen in some cases. Besides Case 43, in which, when the unlike group transfusion was the third in the series instead of the first, a value of 84 was obtained, and Case 57,

3. Keith, N. M.: The Total Circulating Volume of Blood and Plasma in Cases of Chronic Anemia and Leukemia, Am. J. M. Sc. **165**:174-184 (Feb.) 1923.

4. Bock, A. V.: The Constancy of the Volume of the Blood Plasma, Arch. Int. Med. **27**:83-101 (Jan.) 1921.

in which a second transfusion gave a value of 111, there are three other cases. In Case 60, in which a second transfusion gave a value of 63; in Case 49, in which a third transfusion gave a value of 91, which was slowly decreased during the succeeding eleven days, and in Case 112, in which the fifth transfusion in two months gave a blood volume value of 87 c.c. for each kilogram of body weight, this value was maintained. On the whole, these figures, obtained immediately from the later unlike group transfusions, are representative of a cross section of the foregoing studies and indicate that the apparent increase in blood volume, which occurred in certain cases, was probably not due to destruction of the transfused corpuscles, but to their dilution by blood volume increase.

## SUMMARY

The patients who were in the worse condition at the beginning of their treatment had the lower blood volumes. The blood volumes of patients who were made much more comfortable by their transfusions increased, even, in some instances, without a great increase in the cell count for each cubic millimeter, although with the increase in plasma indicated, the total amount of circulating corpuscles was greatly increased. It seems probable that when a patient is not definitely gaining, so far as his hemoglobin is concerned, a decrease in the plasma volume means a fall in the cell count. I am inclined to think that, if a patient's blood destroying mechanism will tolerate an increase in the level of his cell count, an increase in the count with a stationary or even compensatory falling plasma volume may be of advantage, but that if his blood destroying mechanism is so sensitive that it will not tolerate much increase in the level of the blood count, then the increase in plasma volume, causing a total increase in the corpuscle content without greatly raising the level of the cell count, would be helpful. Except in the case of the obese woman, there seems to be a relationship between the throbbing sensations and ringing in the ears, and the low blood volume. Generally, these symptoms disappeared with the establishment of a blood volume of 57 c.c. for each kilogram of body weight.

# THE MECHANISM OF REACTION OF NONSPECIFIC PROTEIN AGENTS IN THE TREATMENT OF DISEASE

## II. THE INFLUENCE OF VARIOUS AGENTS ON THE MOBILIZATION OF BLOOD ANTIBODIES *

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When a suitable foreign protein is carefully administered to a patient suffering from acute or chronic infection, there is no doubt that the patient will give evidence of increased antibody production, measured by agglutinin titer, opsonic index, bacteriolytic activity and methods of complement deviation, as observed and repeatedly confirmed by different workers. Here, however, the clinical findings often fail to measure up to the laboratory expectations.

On the other hand, the possibility of these antibodies, as they are mobilized, playing an important rôle, at least in the ultimate recovery of the patient, cannot be ignored, though the temporary relief of the symptoms might not necessarily depend a great deal on them.

Theories have been advanced to explain the mechanism of serum antibody fluctuation, but the opinions of different observers vary as do the results obtained by them.

Teaque and McWilliams [1] show that the intravenous injection of a suitable dose of killed typhoid bacilli in rabbits causes, within twenty-four hours, a "refractory state" which enables them to withstand a dose of living typhoid that is fatal to a normal rabbit. The serums of these rabbits, however, show a distinct but not a marked bacteriolytic titer four and twenty-four hours, respectively, after such injections. They suggest that blood serum is more bactericidal than lymph, and that the vaccine injection causes a greater flow of bactericidal serum into the lymphoid organs and thus overcomes the local infection.

Culver [2] worked chiefly on gonococcal arthritic patients; ten of them received successive injections of proteose and six received killed gonococci. He was not able to find marked differences in results from these two groups of patients treated, nor did he find marked bactericidal

* From the Department of Bacteriology and Immunology, Graduate School of Medicine of the University of Pennsylvania.

1. Teaque, O., and McWilliams, H. I.: The Bacteriolytic Power of Normal and Immune Rabbit Serum for Typhoid Bacilli and the Intravenous Injection of Vaccine upon the Same, J. Immunol. **2**:167; 185 (Feb.) 1917.

2. Culver, H.: Antibodies in Gonococcal Arthritis, J. Lab. & Clin. Med. **3**:11 (Oct.) 1917.

titer changes in the serums of these patients within twenty-four hours after the administration of the proteins. He thinks that the antibody fluctuation caused by the intravenous injection, which produces a chill, might be explained by the motion of the affected part during the chill, and that it is not due directly to the foreign protein injection.

Bruck [3] has suggested that the protein acts as a toxoid antigen which brings about the sensitization of cell receptors and the overproduction of similar sessile receptors, but which has lacked the necessary stimulus to cause the discharge of these receptors.

Recently, Herrmann,[4] who has investigated this subject more fully, has shown that after the intravenous injection of ascitic fluid and human serum there is definite liberation of specific opsonin agglutinin in rabbits previously sensitized with streptococcus or meningococcus, and markedly so of lysin in those sensitized with sheep erythrocytes, while nonspecific protein has no effect on the rabbits previously sensitized with *Bacillus typhosus*. He concludes that the intravenous injection of foreign protein serves as a stimulus for the liberation of specific antibodies in animals in which the previously injected antigen is unable to cause such a liberation, the insufficiency may lie either in the antigen or in the rabbits.

Larson [5] holds practically the same point of view as does Herrmann. He believes that many bacteria, such as pneumococcus and streptococcus, are imperfect antigens corresponding to the heated tetanus toxin, the toxoid. During the active processes of streptococcic or pneumococcic infection, these organisms act as stimuli to induce the cells to produce the specific antibodies against these specific organisms, but these imperfect antigens do not possess the second stimuli, the "exfoliative stimuli," the stimuli which cause the antibodies to be cast off from the cells into the blood circulation. This second stimulus, as Larson assumes, is supplied by the nonspecific agents, such as vaccines, foreign proteins and proteose. In other words, the antigen which stimulates the cells to produce antibodies is specific, while the agent which causes the cells to throw off the antibodies formed is not necessarily specific. From these premises, Larson draws his conclusion that the injection of foreign proteins enables the organism to throw off the so-called sessile antibodies and get them into the circulation.

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3. Bruck, C.: Experimentelle Beitrage zur Theorie der Immunetat, Ztschr. f. Hyg. u. Infectionskrankh. **46**:48; 176, 1904.

4. Herrmann, S. F.: Liberation of Antibodies by Foreign Protein, J. Infect. Dis. **23**:457 (Nov.) 1918.

5. Larson, W. P.: Principles of Foreign Protein Therapy, Minnesota Med. **2**:332 (Sept.) 1919.

In spite of all these divergent observations and opinions, we may safely assume, in the way Petersen does,[6] that:

> We may expect that if injections are made in patients who have been previously immunized that they will respond with an increase in the antibody titer of the serum; if they are injected during the course of the disease, antibodies which have been formed in the cells but not yet cast off may be "shed," as Larson has suggested, and we may then determine an increase in the serum. On the other hand, if they have been thrown into the circulation during the course of a disease as rapidly as formed, we cannot expect any increase in the titer. We may also assume that with the stimulation of the cellular activity ("plasma-activation") the cell will respond by producing an increased amount of antibodies if they still are capable of such response.

As antibodies, such as antitoxins, opsonins, agglutinins, bacteriolysins, hemolysins and other cytolysins are frequently found in the serums of normal persons and animals, we may be allowed to assume, too, that these antibodies, the "natural receptors," as they are present naturally in the organism, are capable of responding to the stimuli, and should suffer from the same fate, more or less, as those which are artificially produced, after an injection of a suitable dose of appropriate nonspecific protein agent. By using normal, instead of previously sensitized persons and animals as subjects for study, we may eliminate, to some extent, the possible influence which may rise from difference in degrees to which the persons or animals are sensitized. We thus are given a more adequate means to investigate the relative potency of various agents used in proteinotherapy and their selective, if any, influences on different antibodies.

## PURPOSE OF INVESTIGATION

The study of the influence of various nonspecific agents on the thermoregulatory disturbances and the mobilization of leukocytes was given in a previous paper.[7] The present interest has been devoted to a comparative study of the influence of various nonspecific agents on the mobilization of various native antibodies in the serums of normal persons and rabbits, using the same variety of agents as were used in the previous experiments.

## PREPARATION OF AGENTS AND DOSAGE

Eight agents were used, and the size of the dosage depended on the weight of the patient or rabbit. They were as follows:

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6. Petersen, W. F.: Protein Therapy and Nonspecific Resistance, Ed. 1, 1922, p. 67.

7. Ling, C. Y.: Studies of the Mechanism of Reaction of Nonspecific Protein Agents in the Treatment of Disease. I. Influence of Various Agents on Temperature, Arch. Int. Med. **35**:598 (May 15) 1925.

1. Sterile distilled water: 0.1 c.c. per kilogram of weight for human being; 1 c.c. per kilogram of weight for rabbit, given intravenously.

2. Autoserum: 0.5 c.c. per kilogram of weight for human being and rabbit, intravenously.

3. Sterile horse serum: 0.2 c.c. per kilogram of weight for human being and rabbit, given intravenously.

4. Certified milk with a bacterial count of from 7,000 to 10,000, boiled for ten minutes: 0.2 c.c. per kilogram of weight for human being and rabbit, given intramuscularly.

5. Market milk, with bacterial count of from 300,000 to 400,000, boiled for ten minutes: 0.2 c.c. per kilogram of weight for human being and rabbit, given intramuscularly.

6. Crotalin: $\frac{1}{15,000}$ grain per kilogram of weight for human being and $\frac{1}{3,000}$ grain per kilogram of weight for rabbit, dissolved in saline solution and given hypodermically. The material was kindly supplied by Dr. Spangler of Philadelphia.

7. Peptone, a 1 per cent. solution in physiologic sodium chlorid solution, autoclaved for twenty minutes: 0.02 c.c. per kilogram of weight for human being and rabbit, given intravenously.

8. Typhoid vaccine, consisting of 100,000,000 heat killed organisms suspended in saline solution: 0.2 c.c. per kilogram of weight for human being and rabbit, given intravenously.

### ANTIBODIES STUDIED

Three specimens of blood were obtained from each patient or rabbit before and four and twenty-four hours, respectively, after the administration of the agent. The serums obtained were subjected to the following studies:

1. Serum titer of the bactericidal activity against *Bacillus typhosus*.

2. Serum titer of the bactericidal activity against *Staphylococcus aureus*.

3. Bacteriotropic value of the serum in phagocytic activity for *Staphylococcus aureus*.

4. Serum titer of natural hemolysin in hemolytic activity against sheep erythrocytes.

5. Serum titer of complement in hemolytic activity against sheep erythrocytes.

### BACTERIOLOGIC TECHNIC

*Bacteriolytic Titration.*—The method devised by Lacy and Heist,[8] for determining the bactericidal power of the coagulable blood against pneumococcus was principally used in bacteriolytic titration for *B. typhosus*

8. Heist, G. D.: Solis-Cohen, S., and Solis-Cohen, M.: Bactericidal Action of Whole Blood, J. Immunol. **3**:261 (July) 1918.

and *Staphylococcus aureus*. Typhoid suspension was a culture (Rawling's) grown for twenty-four hours in nutrient bouillon at 37.5 C.

In applying the Lacy-Heist method to this laboratory culture, it was found that the coagulable blood of normal persons or rabbits would, in most instances, entirely prohibit the growth of this organism, even when the latter was used in undiluted form. Furthermore, it also was found that the bactericidal titer varies considerably in the serum of each individual subject studied.

For these reasons, the approximate value of the serum of each individual subject was preliminarily estimated by setting up varying dilutions of serum with the standard typhoid suspension (10,000,000,000 per cubic centimeter), using Wright's many stemmed pipet method. After twenty-four hours' incubation, the lowest serum which showed no inhibition of bacterial growth was taken as the fiducial serum dilution, like which, for the regular comparative tests, all those specimens taken from one particular individual subject were prepared.

Each of these three serum dilutions was then set up with the five varying sterngths of the standard typhoid suspension (10,000,000,000 per cubic centimeter), viz., undiluted, 1:5, 1:25, 1:125 and 1:625, following closely the technic as described by Lacy-Heist for pneumococcus.

The bactericidal activity of the serum was measured by the strength of the bacterial suspension in which the growth was prohibited, and the comparative value of the bactericidal serum was expressed in terms of times of the weakest bacterial suspension used (1:625), which is designated as 1. Thus, if 1:125 is the lowest dilution in which the growth is found inhibited, the bactericidal value will be $\frac{1}{125}$ divided by $\frac{1}{625}$, which equals 5.

For *Staphylococcus aureus*, a suspension of 500,000,000 per cubic centimeter was used as standard from which undiluted, 1:5, 1:25, 1:125 and 1:625 dilutions were prepared. Coagulable blood was used, employing the Lacy-Heist method without modification. The bactericidal value was recorded as for typhoid bacillus.

*Bacteriotropic Titration.*—The method described by Evans,[9] as used in the Hygienic Laboratory for standardizing the antimeningococcus serum, was employed in these tests for estimating the bacteriotropic value of the serum in phagocytic activity of leukocytes for *Staphylococcus aureus*. Bacteriotropic serum was prepared in undiluted, 1:5, 1:10 and 1:20 dilutions. Staphylococci, which had been grown for twenty-four hours, were washed off from the agar slants and suspended in Locke's solution (10,000,000,000 per cubic centi-

9. Evans, A. C.: The Tropin Reactions of Antimeningococcus Serum, Bull. Hyg. Lab., U. S. P. H. S., No. 124, p. 43, (Nov.) 1920.

meter). Living leukocytes were secured intrapleurally from a rabbit which had been previously injected with aleuronat emulsion (starch, 3 gm.; aleuronat, 5 gm., and ordinary broth, 100 c.c.). After being thoroughly washed with warm citrate solution and Locke's solution, the leukocytes were suspended in Locke's solution also. Serum dilutions, bacterial suspensions and leukocytic suspensions were all used in 0.2 c.c. amounts each.

Bacterial suspension and serum dilution were first mixed and incubated in a water bath (37.5 C.) for forty-five minutes. The leukocytic suspension was then added, and it was reincubated for another forty-five minutes. The smears were then quickly made from each tube and stained. A leukocyte control which carried in it leukocytic suspension and bacterial suspension and saline solution also was included.

The highest serum dilution with which more than 32 per cent. of the leukocytes showed active phagocytosis was read as titer for the serum. The latter was recorded in terms of its titer dilution only when the number of phagocytic leukocytes counted exceeded at least twice those observed from the leukocyte control tube due to spontaneous phagocytosis.

*Titration of Antisheep Cell Serum Hemolysin.*—The hemolytic system consisted of 1 c.c. of 1:20 guinea-pig complement, 1 c.c. of 2 per cent. sheep cell suspension and a dose of inactivated serum varying from 0.2 c.c., 0.4 c.c., to 1.2 c.c. The one-hour incubation method in a water bath was employed. The smallest amount of serum dilution which brought about complete hemolysis was taken as the hemolytic unit of the serum, and the relative value was recorded in terms of number of units per cubic centimeter of serum tested.

*Titration of Antisheep Cell Serum Complement.*—For these tests, a permanent standard solution of antisheep hemolysin, with its unit previously determined by titrating it against 1 c.c. of 2 per cent. sheep cell suspension and 1 c.c. of 1:20 guinea-pig complement, was used throughout.

Natural antisheep hemolysin, which might be present in the serums tested, was first absorbed and removed by mixing 2 parts of the unheated serum with 8 parts of 2.5 per cent. sheep cell suspension at 0 C. for fifteen minutes, and the supernatant serum dilution, which was then 1:5, secured by centrifugalization.

The hemolytic system for complement titration consisted then of 2 units of standard hemolysin, 1 c.c. of 2 per cent. sheep cell suspension and a dose of antisheep cell hemolysin-free serum dilution (1:5), varying from 0.2 c.c., 0.4 c.c., to 1.6 c.c.

The smallest amount of serum dilution which causes complete hemolysis after one hour's incubation is designated as the complement unit of the serum, and the relative value is recorded in terms of the number of units per cubic centimter of serum.

TABLE 1.—*Influence of Various Agents on Bactericidal Activity of Serums of Normal Human Being and Rabbit*

| Agents | Times Examinations Were Made | Results Obtained with B. Typhosus | | | | Results Obtained with Staphylococcus Aureus | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Rabbit | Human Being | Average | Mean Average* | Rabbit | Human Being | Average | Mean Average* |
| Distilled water | Before injection | 15.0 | 5.0 | 10.0 | 50.0 | 0.5 | 5.0 | 2.8 | 50.0 |
| | 4 hours after injection | 2.5 | 0.5 | 1.5 | 44.5 | 0.5 | 5.0 | 2.8 | 50.0 |
| | 24 hours after injection | 75.0 | 5.0 | 40.0 | 80.0 | 13.0 | 1.0 | 7.0 | 54.2 |
| Autoserum | Before injection | 2.5 | 5.0 | 3.7 | 50.0 | 25.0 | 1.0 | 13.0 | 50.0 |
| | 4 hours after injection | 25.0 | 1.0 | 13.0 | 59.3 | 3.0 | 1.0 | 2.0 | 39.0 |
| | 24 hours after injection | 30.0 | 25.0 | 27.5 | 73.3 | 25.0 | 25.0 | 25.0 | 62.0 |
| Horse serum | Before injection | 40.0 | 25.0 | 32.5 | 50.0 | 5.0 | 5.0 | 5.0 | 50.0 |
| | 4 hours after injection | 45.0 | 5.0 | 25.0 | 42.5 | 2.5 | 1.0 | 1.8 | 46.8 |
| | 24 hours after injection | 40.0 | 25.0 | 32.5 | 50.0 | 43.0 | 25.0 | 34.0 | 79.0 |
| Certified milk | Before injection | 10.0 | 5.0 | 7.5 | 50.0 | 1.0 | 5.0 | 3.0 | 50.0 |
| | 4 hours after injection | 50.0 | 1.0 | 25.5 | 68.0 | 5.0 | 1.0 | 3.0 | 50.0 |
| | 24 hours after injection | 10.0 | 25.0 | 17.5 | 60.0 | 1.0 | 25.0 | 13.0 | 60.0 |
| Market milk | Before injection | 5.0 | 5.0 | 5.0 | 50.0 | 1.0 | 1.0 | 1.0 | 50.0 |
| | 4 hours after injection | 8.0 | 25.0 | 16.5 | 61.5 | 13.0 | 18.3 | 15.7 | 64.7 |
| | 24 hours after injection | 58.0 | 25.0 | 82.0 | 128.0 | 5.0 | 5.0 | 5.0 | 54.0 |
| Crotalin | Before injection | 63.0 | 5.0 | 74.0 | 50.0 | 78.0 | 5.0 | 41.5 | 50.0 |
| | 4 hours after injection | 13.0 | 25.0 | 19.0 | 35.0 | 75.0 | 5.0 | 40.0 | 48.5 |
| | 24 hours after injection | 125.0 | 25.0 | 75.0 | 91.0 | 80.0 | 15.0 | 47.5 | 56.0 |
| Peptone | Before injection | 2.5 | 5.0 | 3.8 | 50.0 | 63.0 | 5.0 | 34.0 | 50.0 |
| | 4 hours after injection | 0.5 | 1.0 | 0.8 | 47.0 | 13.0 | 5.0 | 9.0 | 25.0 |
| | 24 hours after injection | 3.0 | 5.0 | 4.0 | 50.2 | 75.0 | 25.0 | 50.0 | 66.0 |
| Typhoid vaccine | Before injection | 12.0 | 5.0 | 8.5 | 50.0 | 1.0 | 1.0 | 1.0 | 50.0 |
| | 4 hours after injection | 13.0 | 5.0 | 10.0 | 51.5 | 1.0 | 25.0 | 13.0 | 62.0 |
| | 24 hours after injection | 125.0 | 25.0 | 75.0 | 116.5 | 15.0 | 25.0 | 20.0 | 60.0 |
| Control | First examination | 3.0 | 5.0 | 4.0 | 50.0 | 3.0 | 1.0 | 2.0 | 50.0 |
| | Second examination | 3.0 | 5.0 | 4.0 | 50.0 | 3.0 | 1.0 | 2.0 | 50.0 |
| | Third examination | 25.0 | 5.0 | 15.0 | 61.0 | 12.5 | 5.0 | 9.0 | 57.0 |

* The value of bactericidal serums taken before administration is arbitrarily set at 50; the increase or decrease in value of those taken four hours and twenty-four hours after is thus added or subtracted therefrom.

## RESULTS

The results of these experiments and the composite results obtained from both human subjects and rabbits are given in Tables 1, 2 and 3. Their relative values are expressed in Charts 1, 2, 3 and 4. The study may be summarized as follows:

*Influence on Bactericidal Power of Serum for Bacillus Typhosus.*—Apparently all the agents used influence, to some extent, the bactericidal titer within twenty-four hours after the administration. It depends, in the first place, on the nature of the protein agent used. The steady increase of serum titer, after the injections of typhoid vaccine and market milk that is rich in bacterial content, seems to indicate the great potency of some proteins of bacterial origin in producing the nonspecific resistance against typhoid infection. Secondly, the bactericidal serum titer is also undoubtedly influenced by the mere drawing of blood from the animal

and the trauma resulting therefrom, which acts in the same way as a mild form of nonspecific protein agent, as evidenced by the results obtained from human and rabbit controls. Lastly, no two animals react alike; allowances have to be given, of course, to individual differences (Table 1, Chart1).

*Influences on Bactericidal Power of Serum for Staphylococcus Aureus.*—The same results as those with *B. typhosus* are observed so far as the greater potency of bacterial proteins and the phenomena of venesection reactions are concerned. The individual variations are

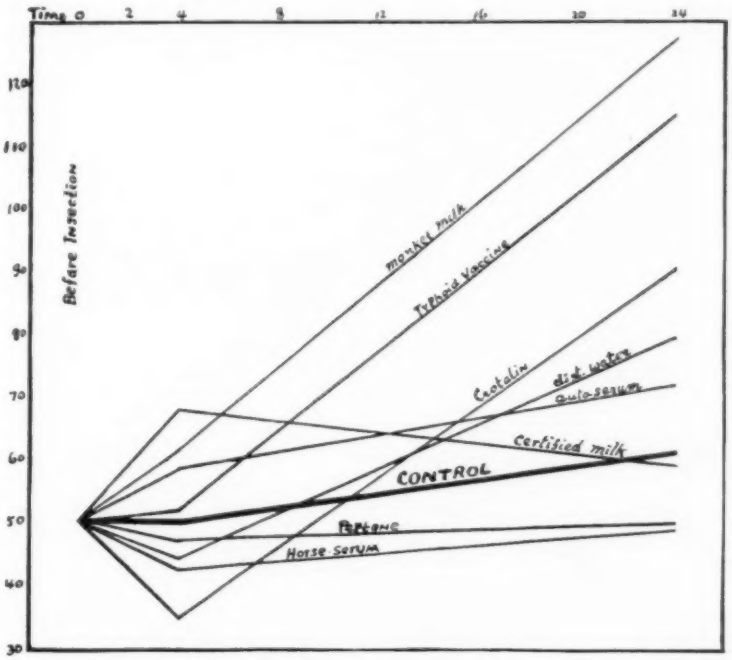


Chart 1.—Composite results of influence of various agents on bactericidal activity of serums of human being and rabbit for *Bacillus typhosus*.

probably the explanation for the results observed in those treated with horse serum and peptone (Table 1, Chart *2*).

*Influence on the Bacteriotropic Content of Serum in Phagocytic Activity for Staphylococcus Aureus.*—The opsonin titer which shows decided increase, as observed four hours after injection, in those treated with peptone, market milk and typhoid vaccine, has the tendency to resume its usual, or normal, opsonin level in about twenty-four hours after administration, while, on the contrary, decided drops of

titer, persisting for more than twenty-four hours, are seen in those treated with autoserum, crotalin and certified milk. Apparently, there is a decided increase of opsonin content in the serum sometimes within the period of four hours after the administration of any of the agents studied. After the maximum of this transient rise of opsonin titer has been reached, it quickly comes down to normal or even to subnormal, in about twenty-four hours after the treatment (Table 2, Chart 3).

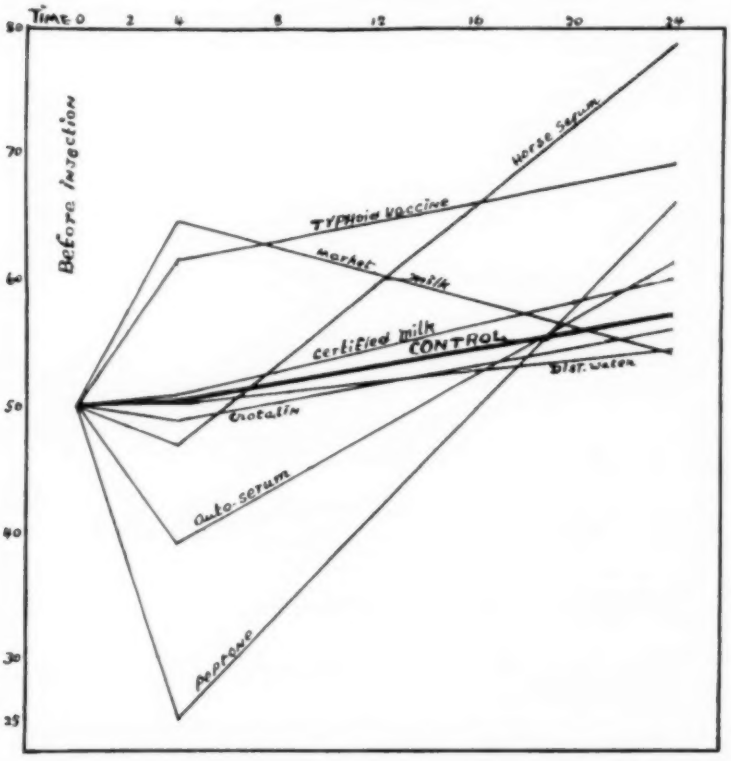


Chart 2.—Composite results of influence of various agents on bactericidal activity of serums of human being and rabbit for *Staphylococcus aureus*.

*Influence on Antisheep Hemolysin and Antisheep Hemolytic Complement.*—The effect of protein treatment on the antisheep hemolysin content of serum is very slight, if any, as shown in these experiments. Not infrequently, the normal serums, either human or rabbit, were found to be practically free from the natural antisheep hemolysin, by the method employed. The fluctuation of complement content, however, is decidedly noticeable and after a fairly constant manner. Apparently, the introduction of one dose of foreign protein has called for the

TABLE 2.—*Influence of Various Agents on the Serum Bacteriotropin in Phagocytic Activity for Staphylococcus Aureus*

| Agents | Times Examinations Were Made | Rabbit | Human Being | Average | Mean Average* |
|---|---|---|---|---|---|
| Distilled water | Before injection | 15.0 | 10.0 | 12.5 | 10.0 |
| | 4 hours after injection | 3.0 | 20.0 | 11.5 | 9.0 |
| | 24 hours after injection | 10.0 | 20.0 | 15.0 | 12.5 |
| Autoserum | Before injection | 7.5 | 20.0 | 13.8 | 10.0 |
| | 4 hours after injection | 3.0 | 20.0 | 11.5 | 7.7 |
| | 24 hours after injection | 2.5 | 14.0 | 8.0 | 4.2 |
| Horse serum | Before injection | 1.0 | 20.0 | 10.5 | 10.0 |
| | 4 hours after injection | 7.5 | 10.0 | 9.0 | 8.5 |
| | 24 hours after injection | 3.0 | 20.0 | 11.5 | 11.0 |
| Certified milk | Before injection | 1.0 | 10.0 | 5.5 | 10.0 |
| | 4 hours after injection | 7.5 | 10.0 | 9.0 | 13.5 |
| | 24 hours after injection | 5.0 | 20.0 | 12.5 | 7.0 |
| Market milk | Before injection | 3.0 | 10.0 | 6.5 | 10.0 |
| | 4 hours after injection | 7.0 | 20.0 | 13.5 | 17.0 |
| | 24 hours after injection | 10.0 | 10.0 | 10.0 | 13.5 |
| Crotalin | Before injection | 12.0 | 10.0 | 11.0 | 10.0 |
| | 4 hours after injection | 17.0 | 5.0 | 11.0 | 10.0 |
| | 24 hours after injection | 6.0 | 5.0 | 5.5 | 4.5 |
| Peptone | Before injection | 3.0 | 5.0 | 4.0 | 10.0 |
| | 4 hours after injection | 12.5 | 10.0 | 11.5 | 17.5 |
| | 24 hours after injection | 5.5 | 10.0 | 8.0 | 14.0 |
| Typhoid vaccine | Before injection | 5.0 | 5.0 | 5.0 | 10.0 |
| | 4 hours after injection | 4.0 | 10.0 | 7.0 | 12.5 |
| | 24 hours after injection | 5.0 | 10.0 | 7.5 | 12.5 |
| Control | First examination | 12.5 | 7.5 | 10.0 | 10.0 |
| | Second examination | 12.0 | 15.0 | 13.5 | 13.5 |
| | Third examination | 13.2 | 10.0 | 11.6 | 11.6 |

* The bacteriotropic value of the serums taken before administration is arbitrarily set at 10; the increase or decrease in value of those examined four hours and twenty-four hours after is thus added or subtracted therefrom.

TABLE 3.—*Influence of Various Agents on Serum Hemolysin Content and Serum Complement Content in Hemolytic Activity for Sheep Erythrocytes*

| | | Serum Hemolysin | | | | Serum Complement | | | |
|---|---|---|---|---|---|---|---|---|---|
| Agents | Times Examinations Were Made | Rabbit | Human Being | Average | Mean Average* | Rabbit | Human Being | Average | Mean Average* |
| Distilled water | Before injection | 5.0 | 2.5 | 3.8 | 5.0 | 2.0 | 1.4 | 1.7 | 5.0 |
| | 4 hours after injection | 5.0 | 2.5 | 3.8 | 5.0 | 1.7 | 1.1 | 1.4 | 4.7 |
| | 24 hours after injection | 5.0 | 2.5 | 3.8 | 5.0 | 2.0 | 1.3 | 1.65 | 4.95 |
| Autoserum | Before injection | 5.0 | ... | 2.5 | 5.0 | 1.0 | 1.3 | 1.15 | 5.0 |
| | 4 hours after injection | 5.0 | ... | 2.5 | 5.0 | 1.0 | 1.3 | 1.15 | 5.0 |
| | 24 hours after injection | 3.5 | ... | 1.8 | 4.3 | 1.1 | 1.7 | 1.4 | 5.25 |
| Horse serum | Before injection | 1.0 | ... | 0.5 | 5.0 | 1.1 | 1.3 | 1.2 | 5.0 |
| | 4 hours after injection | 1.0 | ... | 0.5 | 5.0 | 1.1 | 1.3 | 1.2 | 5.0 |
| | 24 hours after injection | 1.0 | ... | 0.5 | 5.0 | 1.3 | 1.7 | 1.5 | 5.3 |
| Certified milk | Before injection | 2.5 | 2.5 | 2.5 | 5.0 | 1.3 | 1.3 | 1.3 | 5.0 |
| | 4 hours after injection | 2.2 | 2.5 | 2.4 | 4.9 | 1.3 | 1.3 | 1.3 | 5.0 |
| | 24 hours after injection | 2.5 | 2.5 | 2.5 | 5.0 | 1.4 | 1.4 | 1.4 | 5.1 |
| Market milk | Before injection | 3.3 | 2.5 | 2.9 | 5.0 | 1.0 | 1.4 | 1.2 | 5.0 |
| | 4 hours after injection | 3.0 | 2.5 | 2.8 | 4.9 | 0.8 | 1.3 | 1.05 | 4.9 |
| | 24 hours after injection | 3.3 | 2.5 | 2.9 | 5.0 | 1.0 | 1.4 | 1.2 | 5.0 |
| Crotalin | Before injection | 2.0 | ... | 1.0 | 5.0 | 2.0 | 1.0 | 1.5 | 5.0 |
| | 4 hours after injection | 2.0 | ... | 1.0 | 5.0 | 1.7 | 0.8 | 1.25 | 4.75 |
| | 24 hours after injection | 2.0 | ... | 1.0 | 5.0 | 2.0 | 1.0 | 1.5 | 5.0 |
| Peptone | Before injection | 1.0 | 1.0 | 1.0 | 5.0 | 1.1 | 1.1 | 1.1 | 5.0 |
| | 4 hours after injection | 1.0 | 1.0 | 1.0 | 5.0 | 1.0 | 0.8 | 0.9 | 4.8 |
| | 24 hours after injection | 1.0 | 1.0 | 1.0 | 5.0 | 1.1 | 1.0 | 1.05 | 4.95 |
| Typhoid vaccine | Before injection | 0.5 | 2.0 | 1.3 | 5.0 | 1.3 | 1.1 | 1.2 | 5.0 |
| | 4 hours after injection | 0.5 | 2.0 | 1.3 | 5.0 | 1.1 | 1.1 | 1.1 | 4.9 |
| | 24 hours after injection | 0.5 | 2.0 | 1.3 | 5.0 | 1.3 | 1.3 | 1.3 | 5.1 |
| Control | First examination | 2.0 | 0.5 | 1.3 | 5.0 | 1.3 | 0.8 | 1.05 | 5.0 |
| | Second examination | 2.0 | 0.5 | 1.3 | 5.0 | 1.3 | 0.8 | 1.05 | 5.0 |
| | Third examination | 2.0 | 0.5 | 1.3 | 5.0 | 1.6 | 0.8 | 1.2 | 5.15 |

* The serum titer of the serums examined before administration is arbitrarily set at 5 units per cubic centimeter; the increase or decrease at two subsequent examinations is thus added or subtracted therefrom.

sacrifice of the nonspecific serum complement during a period of about four hours directly after the administration, as evidenced by the decided drop of antisheep hemolytic content of serum, shown in these experiments. The complement titer is thence gradually recovered to its original amount, or slightly in excess, in about twenty-four hours.

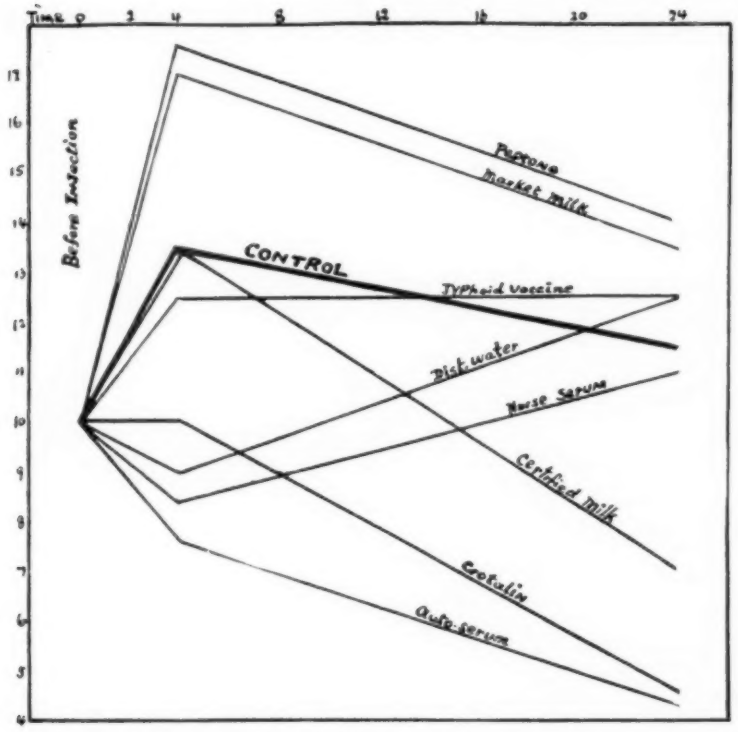


Chart 3.—Composite results of influence of various agents on serum bacteriotropin in phagocytic activity for *Staphylococcus aureus*.

## CONCLUSIONS

1. Natural serum antibodies, including bacteriolysin for *B. typhosus* and *Staphylococcus aureus*, bacteriotropin for *Staphylococcus aureus* in phagocytic activity, and antisheep hemolytic complement, but not antisheep hemolysin, are either mobilized or caused to be "shed" from the organism to some extent, within twenty-four hours, by an injection of any of the following agents: distilled water, autoserum, horse serum, certified milk, market milk, crotalin, peptone and typhoid vaccine.

2. Bacterial proteins, as represented by typhoid vaccine and market milk that is rich in bacteria, are found to be the most efficient agents for

producing a more decided and persistent increase of bacteriolysin against *B. typhosus* and *Staphylococcus aureus*.

3. Peptone and high bacterial count market milk cause an increase of opsonic index in phagocytic activity against *Staphylococcus aureus* to the highest degree.

4. Various agents have no effect on the serum content of natural antisheep hemolysin.

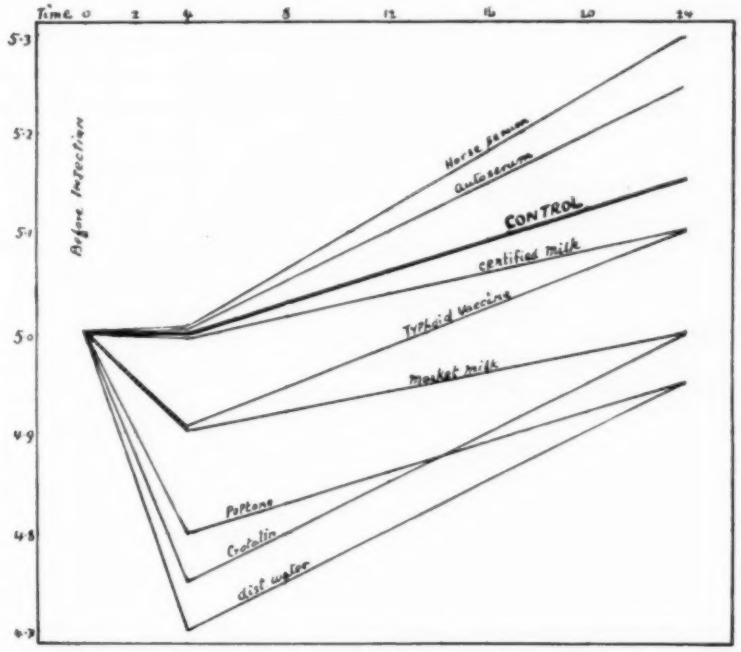


Chart 4.—Composite results of influence of various nonspecific protein agents on serum complement in antisheep erythrocyte hemolytic activity.

5. In general, antisheep hemolytic complement content of serum is reduced after protein treatment, and the reduction persists for more than twenty-four hours, its greatest decrease being sometime between injection and about four hours after, while its recovery to normal or even to slightly above normal level, as is found, in this case, in the serum under the influence of horse serum and autoserum, is about twenty-four hours after the administration.

6. Bleeding the animal and the slight trauma resulting therefrom also will act as a mild form of nonspecific protein agent, which will likewise cause the nonspecific response.

# THE MECHANISM OF REACTION OF NONSPECIFIC PROTEIN AGENTS IN THE TREATMENT OF DISEASE

## III. THE INFLUENCE OF VARIOUS AGENTS ON THE MOBILIZATION OF BLOOD ENZYMES IN NORMAL PERSONS AND IN RABBITS *

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In the blood stream, several proteolytic enzymes are known to occur. These include at least two leukoproteases which are capable of splitting native proteins largely to the proteose stage, and an erepsin-like enzyme freely hydrolyzing proteins from the intermediate stages (albumose and peptone) to the amino-acid forms. These enzymes seem to be derived from the disintegrating but not from living polymorphonuclear leukocytes, and fluctuations in the peripheral leukocyte count are not indicative of the relative titer of the enzyme concentration.[1]

Apart from leukocytes as the chief sources of proteolytic enzymes present in normal blood, small amounts of various digestive enzymes may be derived from gastro-intestinal tracts, the large abdominal organs and from areas altered pathologically if at all. They enter the blood stream possibly by way of the lymph current. These include a tryptase or protease, a polyvalent trypsin-like ferment capable of splitting native proteins all the way down to their lowest split products, if the antiferments are removed, a serum ereptase or peptidase hydrolyzing partly digested proteins to amino-acids and a lipase that splits the fats. That serum contents of these enzymes are readily influenced by diet has been shown by Jobling, Petersen and Eggstein.[2]

In normal blood, these enzymes are present in small amounts, but when foreign proteins are introduced into the system, enzyme activities are found to be excessively increased. Foreign protein may be in the form of living bacteria, as in the case of infection, invading, as they do, the system. It may be in the form of vaccines, toxins or serums, as those are used in immunologic inoculations and in protein therapy. These excessive enzymes are either elaborated by body cells as a defensive response to the protein stimuli or derived from gastro-intestinal tracts,

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1. Petersen, W. F.: The Ferment-Antiferment Balance and Its Relation to Therapeusis, Arch. Int. Med. **20**:515 (Oct.) 1917. Hedin, S. G.: Ueber proteolytische Enzyme im Harn, Ztschr. f. physiol. Chem. **112**:252, 1921.

2. Jobling, J. W.; Petersen, W. F., and Eggstein, A. A.: Serum Ferment and Antiferment After Feeding, J. Exper. Med. **22**:129, 1915.

owing to accelerated lymph flow accompanying the protein shock, or they may be derived from both.[3] The fact cannot be entirely established at present.

These blood ferments, from whatever sources they may be, must have played an important rôle in aiding to digest, destroy and finally to eliminate entirely from the system the noxious materials.

Proteins are known to be more or less toxic only when they are belonging to or have been hydrolyzed to a certain split stage. Various peptones are supposed to be most toxic to the system. Vaughan's belief that bacterial intoxication was due to enzyme digestion of infective materials was based on this. On the other hand, if the enzymes, with which we are richly supplied, are capable of splitting toxic materials further on to nontoxic stages, toxins deliberated would thus be detoxicated as fast as they are formed. The disease foci are thus gradually and favorably influenced in this way, and without leading the patient to a state of undesired intoxication. In connection with this, Petersen and Short, in studying pneumonia that terminates by crisis or by lysis, were able to observe invariably an increase of ereptase titer in the blood, either preceding or accompanying the clinical changes.[4]

Had these facts been established, our success in using nonspecific proteins in the treatment of infectious diseases would depend on the right kind of agents, which would bring about an increase of various blood enzymes and in their desired proportions.

By using normal persons and normal rabbits, free from any detectable disease foci, by taking precautions to avoid the possible effect of diet, and by employing the commonly recognized dosage, a favorable approach is offered to the study of the potencies of various protein agents used to produce an increase in blood enzyme activities.

## PURPOSE OF INVESTIGATION

Comparative studies of the influence of various nonspecific protein agents (given below) on the thermoregulatory center and the mobilization of blood leukocytes and antibodies in normal persons and rabbits were given in two previous papers.[5] The influence of the same agents on the enzyme activities of blood in normal persons and rabbits is to be summarized in this paper.

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3. Jobling, J. W.; Petersen, W. F., and Eggstein, A. A.: The Mechanism of Anaphylactic Shock, Studies on Ferment Action, J. Exper. Med. **22**:401, 1915.

4. Petersen, W. F., and Short, C. A.: On the Relation of the Serum Ereptase (Peptidase) Titer to the Clinical Course in Pneumonia, J. Infect. Dis. **22**:142 (Feb.) 1918.

5. Ling, C. Y.: The Mechanism of Reaction of Nonspecific Protein Agents in the Treatment of Disease. I. Influence of Various Agents on Temperature, Arch. Int. Med. **35**:598 (May 15) 1925. II. Influence of Various Agents on Mobilization of Blood Antibodies, ibid., this issue.

### NONSPECIFIC AGENTS AND DOSAGE

Eight agents and the doses used were the same as used in the previous experiments. They may be recapitulated as follows:

1. Sterile distilled water: 0.1 c.c. per kilogram of weight for human being; 1 c.c. per kilogram of weight for rabbit, given intravenously.

2. Autoserum: 0.5 c.c. per kilogram of weight for human being and rabbit, given intravenously.

3. Sterile horse serum: 0.2 c.c. per kilogram of weight for human being and rabbit, given intravenously.

4. Certified milk with a bacterial count of from 7,000 to 10,000, boiled for ten minutes: 0.2 c.c. per kilogram of weight for human being and rabbit, given intramuscularly.

5. Market milk, with a bacterial count of from 300,000 to 400,000, boiled for ten minutes: 0.2 c.c. per kilogram of weight for human being and rabbit, given intramuscularly.

6. Crotalin: $\frac{1}{15,000}$ grain per kilogram of weight for human being and $\frac{1}{3,000}$ grain per kilogram of weight for rabbit, dissolved in saline solution and given hypodermically. The material was supplied by Dr. Spangler of Philadelphia.

7. Peptone, a 1 per cent. solution in physiologic sodium chlorid solution, autoclaved for twenty minutes: 0.02 c.c. per kilogram of weight for human being and rabbit, given intravenously.

8. Typhoid vaccine, consisting of 100,000,000 heat killed organisms suspended in saline solution: 0.2 c.c. per kilogram of weight for human being and rabbit, given intravenously.

### ENZYMES STUDIED

To avoid the possible effect of diet on the fluctuation of blood enzymes, all examinations were made in the morning before food was given. Untreated individuals used for controls were examined in the same way. Three blood specimens were taken, before, four hours after, and twenty-four hours after treatment. Each serum obtained was subjected to the following enzyme determinations:

1. Degree of the proteolytic activity of serum, in digesting its own native proteins present in the serum.

2. Degree of the peptolytic activity of serum in digesting a given amount of peptone solution.

3. Degree of lipolytic activity of serum in digesting a given amount of ethyl butyrate.

TECHNIC

*Proteolytic Technic.*—The technic employed in these enzyme tests was principally that described by Petersen. The method employed for studying the proteolytic activity is briefly as follows: 1.5 c.c. of hemoglobin free serum and 3 c.c. of chloroform were thoroughly mixed in a graduated test tube (3 cm. in diameter). Chloroform was added here to hold in check the serum anti-enzyme action. A control tube that carried in it 1.5 c.c. of serum, with 2 drops of toluene added as preservative, was inactivated at 60 C. for thirty minutes. This control tube and the sample tube were incubated at 37.5 C. for forty-eight hours, tubes being shaken at regular intervals. One cubic centimeter of a mixture of 10 per cent. acetic acid plus 20 per cent. sodium chlorid solution was then added to each tube. To remove the chloroform, the tube was gently heated in a water bath. When the chloroform was entirely evaporated from the sample tube, 4 c.c. of water was added to each tube, and the temperature was raised to the boiling point, and kept at such for ten minutes. When cooled, sufficient water was added to make up a total volume of 15 c.c. Coagulable proteins were filtered off. Ten cubic centimeters of filtrate, equivalent to 1 c.c. of serum, was then secured. The total nitrogen content was determined according to Kjeldahl's method. The difference in milligrams of uncoagulable nitrogen between 100 c.c. of sample serum and 100 c.c. of control serum expresses the proteolytic enzyme units.

*Peptolytic Technic.*—In five test tubes, 1 c.c. of the following serum dilutions were prepared: Undiluted, 1:2, 1:4, 1:8 and 1:16. To each tube, 1 c.c. of 4 per cent. peptone solution and 1 c.c. of toluene were added. They were then incubated at 37.5 C. for eighteen hours. Bromin water was then introduced into each tube, drop by drop, until the pink color appeared. The reciprocal of the highest serum dilution that shows the pink color expresses the peptolytic enzyme units.

*Lipolytic Technic.*—One cubic centimeter of serum, 1 c.c. of ethyl butyrate, 0.5 c.c. of toluene, and a sufficient amount of physiologic sodium chlorid solution to bring the total volume up to 10 c.c. were mixed thoroughly in a flask. A serum control and a peptone control were also included. The tubes were incubated at 37 C. for four hours. Twenty-five cubic centimeters of neutral alcohol was then added to each flask. The acidity developed was titrated to faint pink with hundredth normal sodium hydroxid, using phenolsulphonephthalein solution as indicator. After the result obtained is deducted from the control, the number in cubic centimeters of hundredth normal sodium hydroxid, used to neutralize the acidity developed by 1 c.c. of serum from 1 c.c. of ethyl butyrate, expresses the lipolytic units.

TABLE 1.—*Influence of Various Agents on Serum Proteolytic Enzymes*

| Agents | Times Examinations Were Made | Rabbit | Human Being | Average | Mean Average* |
|---|---|---|---|---|---|
| Distilled water | Before injection | 3.5 | 2.1 | 2.8 | 4.0 |
| | 4 hours after injection | 2.1 | 0.7 | 1.4 | 2.6 |
| | 24 hours after injection | 6.3 | 1.4 | 3.9 | 5.1 |
| Autoserum | Before injection | 4.5 | 3.5 | 4.0 | 4.0 |
| | 4 hours after injection | 1.4 | 2.7 | 2.1 | 2.1 |
| | 24 hours after injection | 3.5 | 2.4 | 3.0 | 3.0 |
| Horse serum | Before injection | 1.4 | 2.1 | 1.8 | 4.0 |
| | 4 hours after injection | 1.4 | 3.5 | 2.5 | 4.7 |
| | 24 hours after injection | 1.1 | 1.4 | 1.3 | 3.5 |
| Certified milk | Before injection | 1.4 | 1.4 | 1.4 | 4.0 |
| | 4 hours after injection | 1.4 | 0.7 | 1.1 | 3.7 |
| | 24 hours after injection | 0.7 | 2.8 | 1.8 | 4.4 |
| Market milk | Before injection | 2.3 | 2.8 | 2.6 | 4.0 |
| | 4 hours after injection | 3.7 | 1.4 | 2.6 | 4.0 |
| | 24 hours after injection | 4.2 | 2.8 | 3.5 | 4.9 |
| Crotalin | Before injection | 14.0 | 0.7 | 7.4 | 4.0 |
| | 4 hours after injection | 12.4 | 0.8 | 6.6 | 3.2 |
| | 24 hours after injection | 14.1 | 1.4 | 7.8 | 4.4 |
| Peptone | Before injection | 3.4 | 2.8 | 3.1 | 4.0 |
| | 4 hours after injection | 5.0 | 5.6 | 5.3 | 6.2 |
| | 24 hours after injection | 2.8 | 2.8 | 2.8 | 3.7 |
| Typhoid vaccine | Before injection | 7.0 | 1.4 | 4.2 | 4.0 |
| | 4 hours after injection | 5.6 | 3.5 | 4.6 | 4.4 |
| | 24 hours after injection | 2.8 | 0.7 | 1.8 | 1.6 |
| Control | First examination | 2.8 | 3.4 | 3.1 | 4.0 |
| | Second examination | 2.0 | 1.8 | 1.9 | 2.8 |
| | Third examination | 3.0 | 4.0 | 3.5 | 4.4 |

* The proteolytic activity expressed in terms of units, obtained from the specimen taken before injection, is arbitrarily set at 4; the increase or decrease at two subsequent examinations is added to or subtracted from it.

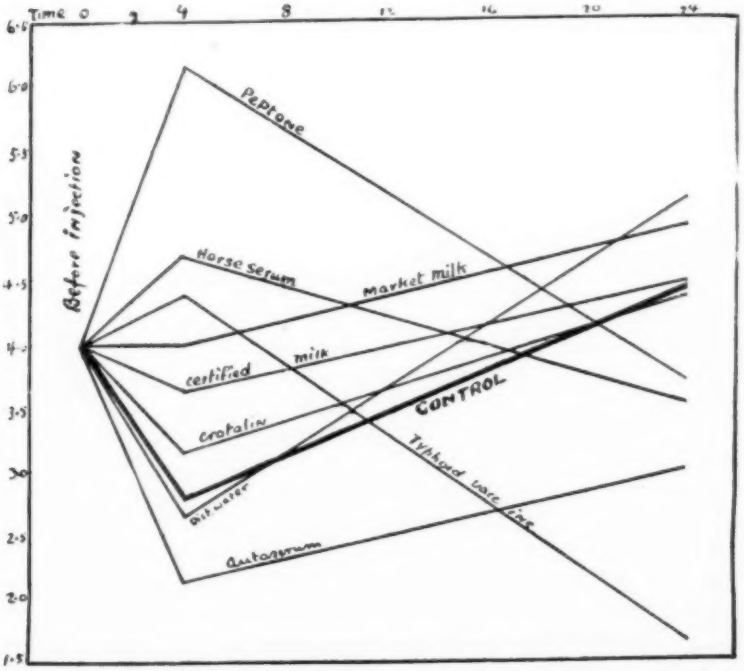


Chart 1.—Composite results of influence of various agents on proteolytic enzymes in blood of human being and rabbit.

## RESULTS

The composite results of these experiments are given in Tables 1 and 2 and in Charts 1, 2 and 3. A brief survey of this study may be summarized as follows:

*Influence on Proteolytic Activities.*—The proteolytic enzymes acted well but slowly at 37.5 C.; incubation for not less than forty-eight hours will insure better results. Practically all the agents used influenced fluctuation of protease content of blood some time between four and twenty-four hours after the injection. Peptone induced a marked and an early response, while market milk and distilled water produced a slower but more persistent increase (Table 1 and Chart 1).

*Influence on Peptolytic Activities.*—The relative values of peptolytic activities obtained by the method employed were only approximately but not absolutely accurate, as inconsistent readings were not infrequently found to occur. As a whole, however, they did definitely indicate a steady increase of peptidase titer in about, or more than, twenty-four hours after injection, while the initial depression was prolonged to about four hours after injection (Table 2 and Chart 2).

TABLE 2.—*Influence of Various Agents Mobilization of Peptolytic and Lipolytic Enzymes (Human)*

| | | Peptolytic Activity | | Lipolytic Activity | |
|---|---|---|---|---|---|
| Agents | Times Examinations Were Made | Units | Mean Average* | Units | Mean Average* |
| Distilled water | Before injection | 8.0 | 10.0 | 11.0 | 10.0 |
| | 4 hours after injection | 8.0 | 10.0 | 9.0 | 8.0 |
| | 24 hours after injection | 12.0 | 14.0 | 10.0 | 9.0 |
| Autoserum | Before injection | 8.0 | 10.0 | 11.0 | 10.0 |
| | 4 hours after injection | 4.0 | 6.0 | 8.0 | 7.0 |
| | 24 hours after injection | 12.0 | 14.0 | 10.5 | 9.5 |
| Horse serum | Before injection | 8.0 | 10.0 | 7.0 | 10.0 |
| | 4 hours after injection | 10.0 | 12.0 | 9.0 | 12.0 |
| | 24 hours after injection | 14.0 | 16.0 | 6.0 | 9.0 |
| Certified milk | Before injection | 14.0 | 10.0 | 5.6 | 10.0 |
| | 4 hours after injection | 14.0 | 10.0 | 6.0 | 10.4 |
| | 24 hours after injection | 20.0 | 16.0 | 6.5 | 10.9 |
| Market milk | Before injection | 14.0 | 10.0 | 6.0 | 10.0 |
| | 4 hours after injection | 16.0 | 12.0 | 6.0 | 10.0 |
| | 24 hours after injection | 20.0 | 16.0 | 7.0 | 11.0 |
| Crotalin | Before injection | 14.0 | 10.0 | 8.0 | 10.0 |
| | 4 hours after injection | 12.0 | 8.0 | 7.0 | 9.0 |
| | 24 hours after injection | 20.0 | 16.0 | 7.0 | 9.0 |
| Peptone | Before injection | 18.0 | 10.0 | 6.0 | 10.0 |
| | 4 hours after injection | 14.0 | 6.0 | 5.0 | 9.0 |
| | 24 hours after injection | 24.0 | 16.0 | 7.0 | 11.0 |
| Typhoid vaccine | Before injection | 8.0 | 10.0 | 6.0 | 10.0 |
| | 4 hours after injection | 2.0 | 4.0 | 7.0 | 11.0 |
| | 24 hours after injection | 14.0 | 16.0 | 5.0 | 9.0 |
| Control | First examination | 8.0 | 10.0 | 8.0 | 10.0 |
| | Second examination | 6.0 | 8.0 | 7.2 | 9.2 |
| | Third examination | 10.0 | 12.0 | 8.2 | 10.2 |

* The peptolytic or lipolytic units obtained from specimens obtained before injection are arbitrarily set at 10; the increase or decrease at two subsequent examinations is added to or subtracted from it.

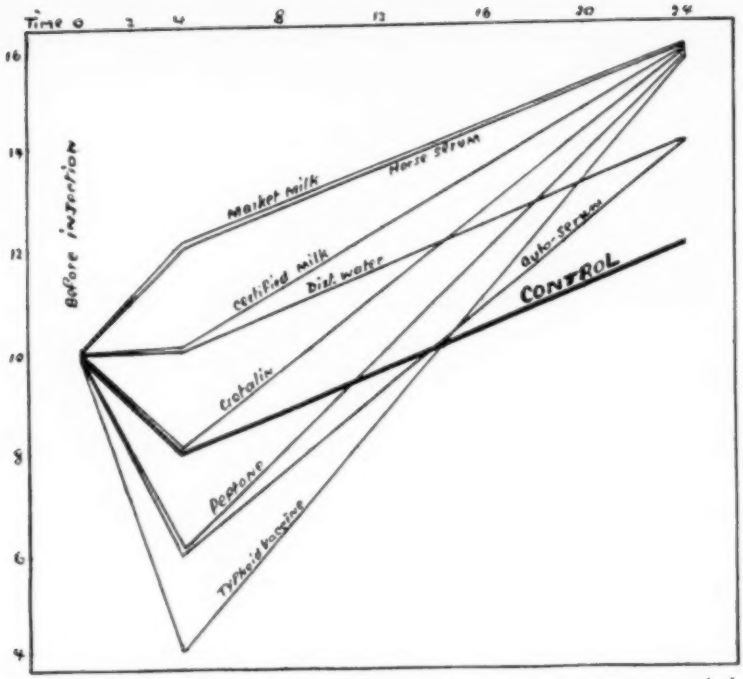


Chart 2.—Composite results of influence of various agents on peptolytic enzymes in blood of human being and rabbit.

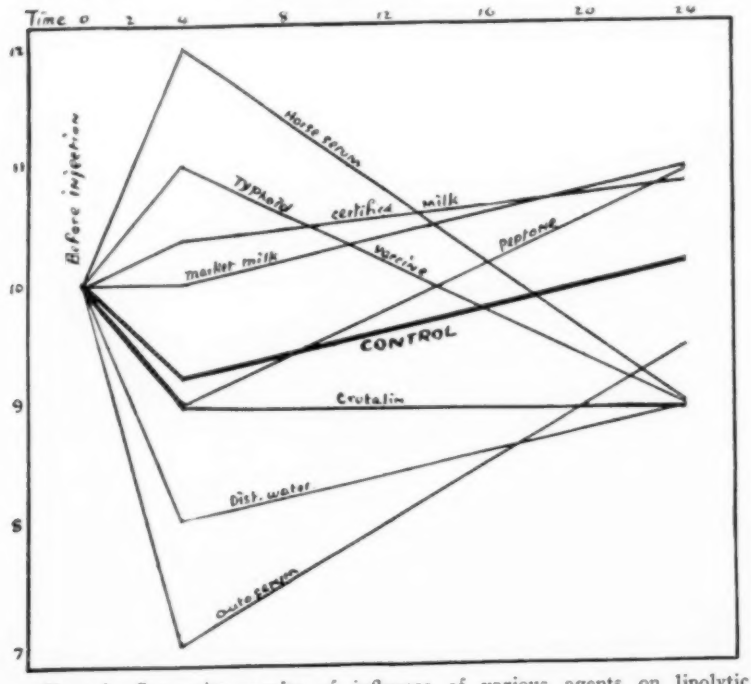


Chart 3.—Composite results of influence of various agents on lipolytic enzymes in blood of human being.

*Influence on Lipolytic Activities.*—The influence of various agents on lipolytic enzymes was the same as on proteolytic ones as far as their inconsistency and irregularity were concerned. Horse serum and typhoid vaccine induced a quicker lipase response in about four hours after administration, while slower but more persistent increases were found in those inoculated with market milk, certified milk and peptone solution (Table 2 and Chart 3).

## CONCLUSIONS

1. Proteolytic, peptolytic and lipolytic serum titers of normal blood are readily influenced by an injection of the following protein agents: distilled water, autoserum, horse serum, certified milk, market milk, crotalin, peptone and typhoid vaccine.

2. A sharp and quick response of proteolytic activity in the serum follows an injection of peptone solution, and of horse serum, while a steady and persistent increase follows an injection of any of the following agents: market milk, distilled water and certified milk, named in the order of their potencies.

3. It seems that there is a steady increase of peptidase titer in the serum, approaching its maximum at about twenty-four hours after an injection of any of the agents used. A decided drop of peptidase titer follows treatment with typhoid vaccine, autoserum and peptone, some time within four hours after treatment.

4. Horse serum and typhoid vaccine produce a sharp and quick response of increased lipolytic serum titer, but market milk, certified milk and peptone produce a steady increase of the serum lipase, persistent for more than twenty-four hours.

5. Bleeding the animal, and the slight trauma resulting therefrom, likewise influence a slight fluctuation of various enzyme contents of the blood.

# HOURLY HEMOGLOBIN VARIATIONS IN ANEMIAS *

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The variations that may occur in the percentage of hemoglobin in a normal man during the day have been shown to be considerable.[1] As much as 26 per cent. has been reported in the course of two hour observations. Numerous factors contribute to this phenomenon.

The question of variations not only in the percentage of hemoglobin but in the color index, red cell volume and plasma cell ratio over two hour periods has been investigated in a series of sixteen cases of anemia. Of these, ten were of the pernicious or addisonian type. The blood was obtained by venipuncture at two hour intervals, beginning at 8 a. m. and terminating at 6 p. m. On each specimen, a red cell enumeration, the hemoglobin percentage by oxygen capacity (Van Slyke) and Dare methods, and plasma cell ratio were determined. From these data, not only the color index and the red cell volume, but also the size of the average cell and the amount of hemoglobin it carried, were readily calculated.

## TECHNIC

*Collection of Blood.*—The patients were not restricted as to food or fluids. Eight cubic centimeters of blood was taken, at 8, 10 and 12 a. m., 2, 4 and 6 p. m., by vena puncture. Clotting was prevented by crystals of potassium oxalate. All determinations were carried out by the same person.

*Blood Counts.*—The average of four blood counts was taken. The hemoglobin estimation was made: (*a*) by the oxygen capacity method (Van Slyke), and (*b*) by a standardized Dare hemoglobinometer To take the plasma cell ratios, hematocrits were made from glass tubing of equal bore. These, after being filled with blood, were centrifugated for thirty minutes at 3,000 revolutions per minute. The proportion of plasma to cells was accurately measured by a millimeter rule.

*Hemoglobin Variations.*—The largest individual variation of the hemoglobin as estimated by the oxygen capacity method was 11.2 per cent. In two cases it was over 7 per cent., but the average of all sixteen cases was only 5.1 per cent. Taking the secondary anemias alone, it was only 4 per cent. This contrasts with those figures recently pub-

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* From the department of metabolism of the Montreal General Hospital.

1. Rabinowitch, I. M., and Strean, G.: Hemoglobin Content of Red Blood Cells in Relation to Their Surface Area, Arch. Int. Med. **34**:124 (July) 1924. Rabinowitch, I. M.: J. Lab. & Clin. Med. **9**:120 (Nov.) 1923.

lished for the normal men in which two cases showed variations of 26 per cent., and four were between 15 and 20 per cent., the average for the series (twenty cases) being 12.4 per cent. The percentage of hemoglobin, as recorded by the oxygen capacity method, was in all instances higher than that of the Dare. In one case, the maximum variation was plus 20.2 per cent., and in four cases between 15 and 20 per cent. The

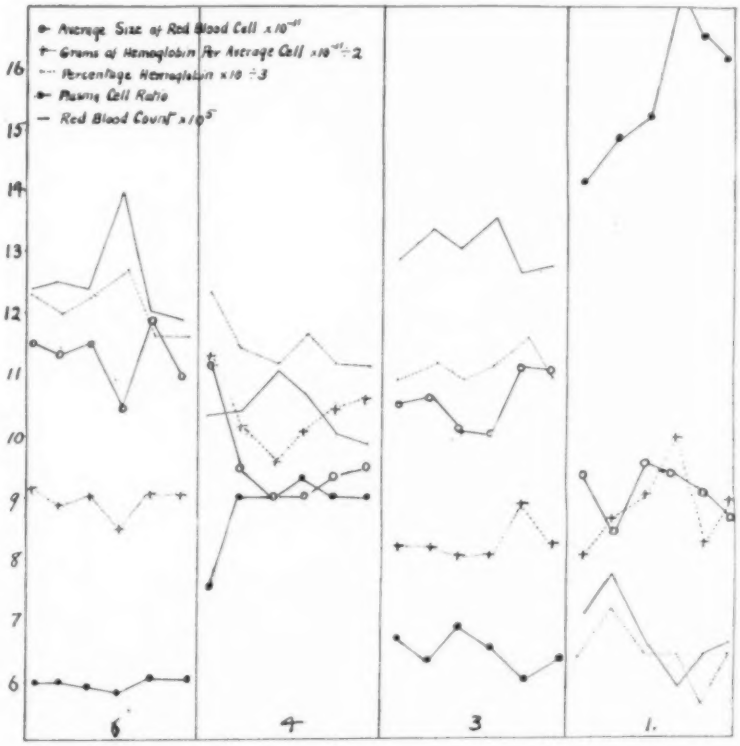


Chart 1

average positive variation for all cases was plus 13 per cent. The Dare hemoglobinometers chosen were of the standardized type used in the medical wards of this hospital.

This discrepancy emphasizes the need for a uniform standard for hemoglobin estimation. The more accurate method of Van Slyke seems to fit in better with the clinical conditions of the patient. The color indexes as calculated by each method also are against the Dare. In Case 1, unquestionably of the pernicious type, the color index would be

only 0.8 by the Dare method, but 1.5 by the Van Slyke method. The same is true of Cases 3, 8 and 10. Table 1 shows a comparison of the indexes as calculated from both methods. Table 2 records the observations made on the hemoglobin, the maximum variations and the average red cell enumeration for each case.

*Size and Hemoglobin of Red Blood Cell.*—The average size of the red blood cell was obtained by dividing the volume of cells per hundred cubic centimeters of blood (hematocrit) by the number of cells in the same volume (red cell enumeration). The amount of hemoglobin in grams per cell was found by dividing the hemoglobin per hundred

TABLE 1.—*Comparison of Indexes as Calculated by Oxygen Capacity and Dare Methods*

| Case | Red Cell Count | Hemoglobin by Oxygen Capacity Method | Gm. of Hemoglobin per 100 C.c. Blood | Index | Index by Dare | Plasma Ratio Cell Volume | Volume of Cells 100 C.c. Blood | Volume of Cell $\times 10^{-11}$ | Grams of Hemoglobin per Cell $\times 10^{-11}$ |
|---|---|---|---|---|---|---|---|---|---|
| Pernicious anemia | | | | | | | | | |
| 1 | 670,000 | 21.2 | 2.97 | 1.5 | 0.8 | 15.4 | 6.0 | 9.0 | 4.4 |
| 2 | 853,000 | 29.0 | 4.05 | 1.6 | 1.2 | 9.0 | 9.2 | 11.0 | 4.7 |
| 3 | 1,250,000 | 37.0 | 5.18 | 1.6 | 0.9 | 6.4 | 11.7 | 10.5 | 4.1 |
| 4 | 1,046,000 | 38.4 | 5.38 | 1.8 | 1.2 | 8.8 | 9.9 | 9.5 | 5.1 |
| 5 | 1,210,000 | 40.2 | 5.60 | 1.6 | 1.1 | 6.5 | 13.1 | 10.9 | 4.6 |
| 6 | 1,250,000 | 40.3 | 5.64 | 1.5 | 1.1 | 5.9 | 4.0 | 11.2 | 4.4 |
| 7 | 1,260,000 | 45.4 | 6.37 | 1.7 | 1.4 | 5.0 | 16.4 | 12.9 | 5.0 |
| 8 | 1,950,000 | 51.0 | 7.14 | 1.2 | 0.9 | 4.2 | 19.3 | 9.9 | 3.6 |
| 9 | 1,600,000 | 59.1 | 8.27 | 1.7 | 1.4 | 3.6 | 21.6 | 13.5 | 5.1 |
| 10 | 3,000,000 | 59.4 | 8.31 | 0.95 | 0.7 | 2.7 | 27.0 | 8.8 | 2.7 |
| Average | | | | | | | | 10.7 | 4.4 |
| Secondary anemia | | | | | | | | | |
| 11 | 2,780,000 | 28.7 | 4.01 | 0.51 | 0.3 | 6.8 | 12.2 | 4.5 | 1.4 |
| 12 | 2,380,000 | 34.6 | 4.84 | 0.72 | 0.4 | 6.2 | 13.5 | 5.6 | 2.0 |
| 13 | 2,010,000 | 39.3 | 5.50 | 0.72 | 0.7 | 6.5 | 13.0 | 6.4 | 2.6 |
| 14 | 3,600,000 | 45.8 | 6.40 | 0.62 | 0.5 | 3.2 | 24.0 | 6.4 | 1.7 |
| 15 | 3,970,000 | 46.4 | 6.46 | 0.58 | 0.4 | 3.2 | 23.5 | 6.0 | 1.6 |
| 16 | 3,330,000 | 57.5 | 7.20 | 0.77 | 0.6 | 3.2 | 23.6 | 7.0 | 2.1 |
| Average | | | | | | | | 5.95 | 1.9 |

cubic centimeters of blood by the number of cells, as in the former case. The figures, obtained in both the primary and secondary types of anemia, correspond closely with those reported by Haden.[2] The mean values for the average size of the cell were somewhat smaller, and for the grams of hemoglobin per cell a trifle greater, than those found by the same author. Thus, the average size of the cell in the ten cases of pernicious type was $10.7 \times 10^{-11}$. Haden gives the average of twenty cases as $12.8 \times 10^{-11}$. The average hemoglobin content of the cell was $4.4 \times 10^{-11}$, as compared with $3.75 \times 10^{-11}$ (Haden) (Table 1).

2. Haden, R. L.: Acute Criteria for Differentiating Anemias, Arch. Int. Med. **31**:766 (May) 1923.

*Hourly Variations in Red Cells.*—In the more severe grades of anemia dealt with, the numerical values of the red cells showed much less tendency to vary than in the normal case. The greatest variation noted was 501,000, but in only a few instances was it over a quarter million. These were, however, sufficient to account for appreciable differences in the average size of the cell. Thus, in one case, Case 7,

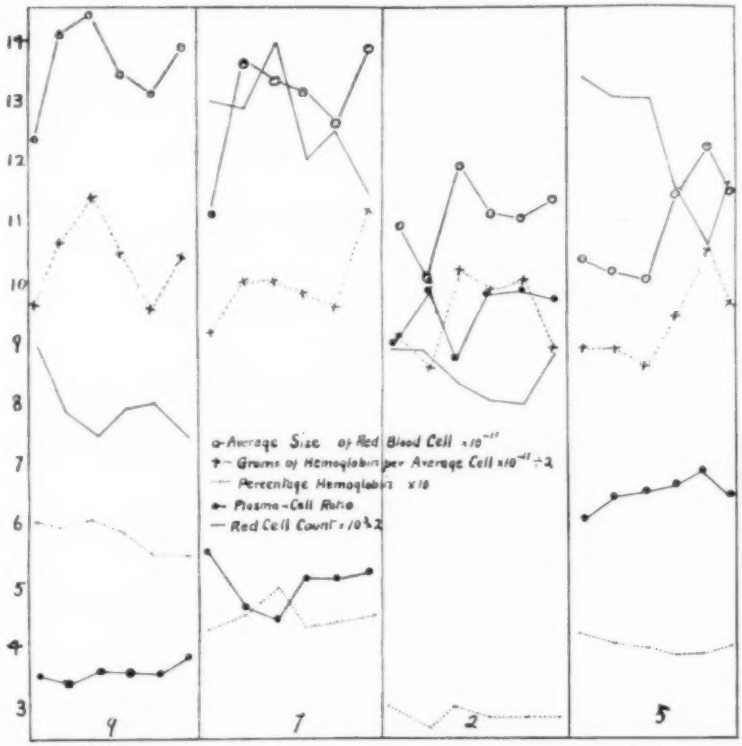


Chart 2

the average size of the cell, as determined at 8 a. m., was $11.1 \times 10^{-11}$, while, two hours later, it was $13.6 \times 10^{-11}$, or a difference of $2.5 \times 10^{-11}$, which means an increase of 18 per cent. The average variations for the primary anemias was $1.69 \times 10^{-11}$ for the secondary $0.88 \times 10^{-11}$. A similar and corresponding variation was noted in the grams of hemoglobin per cell (Table 3). As the average size of the cell increased, the amount of hemoglobin showed a similar rise. This fact is well brought out by the parallelism of the curves of Charts

TABLE 2.—*Two Hour Variations in Hemoglobin*

| | 8 a. m. | | 10 a. m. | | 12 m. | | 2 p. m. | | 4 p. m. | | 6 p. m. | | Maximum Variation Oxygen Capacity Method | Maximum Variation Dare | Maximum Oxygen Capacity over Dare Variation | Average Blood Count | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Case | Oxygen Capacity | Dare | Oxygen Capacity | Dare | Oxygen Capacity | Dare | Oxygen Capacity | Dare | Oxygen Capacity | Dare | Oxygen Capacity | Dare | | | | | Diagnosis |
| 1 | 21.2 | 10 | 24.2 | 14 | 21.2 | 10 | 21.2 | 10 | 18.6 | 11 | 21.2 | 11 | 5.6 | 4.0 | +11.2 | 670,000 | Pernicious anemia |
| 2 | 29.9 | 24 | 27.4 | 21 | 30.7 | 23 | 28.7 | 18 | 28.7 | 20 | 28.7 | 21 | 3.3 | 6.0 | +10.7 | 853,000 | Pernicious anemia |
| 3 | 36.2 | 27 | 37.5 | 27 | 36.2 | 28 | 37.5 | 29 | 38.7 | 28 | 36.2 | 27 | 2.5 | 2.0 | +10.5 | 1,250,000 | Pernicious anemia |
| 4 | 41.2 | 26 | 38.0 | 22 | 37.5 | 28 | 39.2 | 21 | 37.5 | 29 | 37.5 | 28 | 3.7 | 8.0 | +20.2 | 1,040,000 | Pernicious anemia |
| 5 | 42.5 | 30 | 41.2 | 30 | 40.0 | 29 | 38.7 | 28 | 38.0 | 28 | 40.0 | 29 | 4.5 | 2.0 | +12.5 | 1,270,000 | Pernicious anemia |
| 6 | 41.2 | 29 | 40.0 | 31 | 41.2 | 30 | 42.5 | 33 | 38.7 | 31 | 38.7 | 30 | 3.7 | 4.0 | +12.2 | 1,250,000 | Pernicious anemia |
| 7 | 43.2 | 34 | 46.2 | 40 | 50.0 | 38 | 43.2 | 36 | 43.7 | 34 | 46.2 | 36 | 6.8 | 6.0 | + 9.2 | 1,260,000 | Pernicious anemia |
| 8 | 53.8 | 40 | 52.5 | 36 | 51.3 | 42 | 48.7 | 35 | 51.3 | 38 | 48.7 | 39 | 5.1 | 7.0 | +16.5 | 1,960,000 | Pernicious anemia |
| 9 | 61.3 | 54 | 60.0 | 43 | 61.3 | 49 | 59.3 | 47 | 56.3 | 49 | 56.3 | 45 | 5.0 | 11.0 | +17.0 | 1,600,000 | Pernicious anemia |
| 10 | 61.3 | 47 | 58.8 | 41 | 65.0 | 40 | 56.3 | 50 | 61.3 | 49 | 53.8 | 49 | 11.2 | 10.0 | +15.0 | 3,060,000 | Pernicious anemia |
| 11 | 31.2 | 23 | 28.7 | 19 | 31.2 | 23 | 28.7 | 20 | 26.2 | 18 | 26.2 | 21 | 5.0 | 5.0 | + 9.7 | 2,780,000 | Syphilitic anemia |
| 12 | 33.7 | 20 | 33.7 | 25 | 33.7 | 23 | 36.2 | 24 | 36.7 | 21 | 33.7 | 23 | 3.0 | 5.0 | +15.7 | 2,380,000 | Uterine hemorrhage |
| 13 | 58.7 | 28 | 40.0 | 29 | 37.5 | 28 | 38.7 | 29 | 41.0 | 29 | 40.2 | 27 | 3.5 | 2.0 | +13.2 | 2,010,000 | Uterine hemorrhage |
| 14 | 48.7 | 44 | 46.2 | 43 | 46.2 | 47 | 42.5 | 46 | 46.2 | 38 | 45.0 | 39 | 6.2 | 8.0 | + 8.2 | 3,060,000 | Uterine hemorrhage |
| 15 | 45.0 | 38 | 43.7 | 38 | 47.5 | 36 | 48.7 | 41 | 46.2 | 36 | 46.2 | 37 | 5.0 | 5.0 | +11.5 | 3,970,000 | Chronic nephritis |
| 16 | 46.2 | 40 | 47.5 | 43 | 53.8 | 43 | 53.8 | 39 | 53.8 | 40 | 53.8 | 40 | 7.6 | 4.0 | +14.8 | 3,330,000 | Uterine hemorrhage |
| | Average variation by oxygen capacity method | | | | | | | | | | | | 5.1 | | | | |
| | Average Variation by Dare Method | | | | | | | | | | | | | 5.5 | | | |
| | Percentage increase by oxygen capacity over Dare method (average) | | | | | | | | | | | | | | +13.0 | | |

1 to 4, representing the factors in question. As was to be expected, the larger the cell, the more hemoglobin it carried. Reduced to unit volume, however, the amount was fairly constant. These figures, in themselves, are of paramount importance only in that they test the accuracy of the red cell enumerations, which, it must be admitted, is the one factor in which the personal equation is liable to enter most.

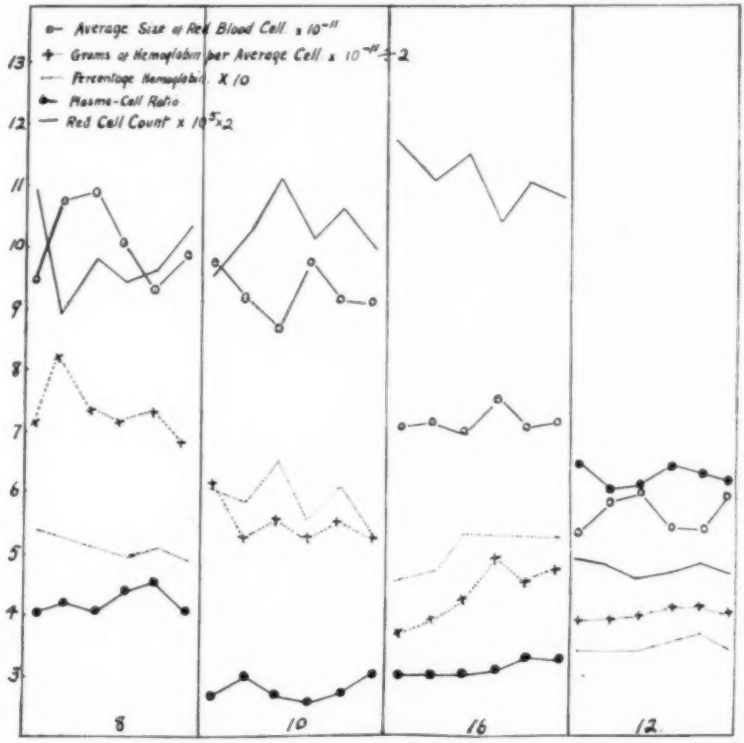


Chart 3

Were the variations artificial, no such parallelism would be obtained, because each curve is dependent on factors other than the red cell count.

### RELATION OF HEMOGLOBIN TO CELL SURFACE IN ANEMIA

Former experiments carried out in this laboratory on the non-anemic patients tend to suggest that the hemoglobin is distributed on the surface of the erythrocyte and not, as is usually taught, within the cell itself. They show that, when the total cell volume is constant, varia-

tions in the amount of hemoglobin in that volume, or vice versa, can best be explained by assuming that the hemoglobin is distributed on the surface of the cell. Thus, with an increase in the number of cells (smaller in size), the total volume remaining constant, there results an increased surface area (smaller cells), hence a corresponding increase in the hemoglobin carrying capacity. If this hypothesis is correct, it appears reasonable to assume that it should hold in anemia. A demonstration that such a law either does or does not obtain in cases of anemia would tend to strengthen or negate this hypothesis.

TABLE 3.—*Two Hour Variations in Grams of Hemoglobin Per Cell*

| Primary Anemia | | | | | | | | Secondary Anemia | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Volume of Red Cell × $10^{-11}$ | | | | | | Maximum | | Volume of Red Cell × $10^{-11}$ | | | | | | Maximum |
| Case | 8 a. m. | 10 a. m. | 12 m. | 2 p. m. | 4 p. m. | 6 p. m. | Variation | Case | 8 a. m. | 10 a. m. | 12 m. | 2 p. m. | 4 p. m. | 6 p. m. | Variation |
| 1 | 9.3 | 8.3 | 9.5 | 9.3 | 9.0 | 8.6 | 1.2 | 11 | 4.6 | 4.1 | 4.8 | 4.7 | 4.6 | 4.5 | 0.7 |
| 2 | 10.8 | 10.0 | 11.8 | 11.1 | 11.0 | 11.3 | 1.3 | 12 | 5.3 | 5.8 | 6.0 | 5.4 | 5.4 | 5.9 | 0.7 |
| 3 | 10.5 | 10.6 | 10.1 | 10.0 | 11.1 | 11.0 | 1.1 | 13 | 6.5 | 6.3 | 5.5 | 6.5 | 6.7 | 6.9 | 1.4 |
| 4 | 11.2 | 9.5 | 9.0 | 9.0 | 9.3 | 9.5 | 2.2 | 14 | 6.2 | 6.5 | 6.6 | 6.0 | 6.8 | 6.6 | 0.8 |
| 5 | 10.3 | 10.2 | 10.0 | 11.4 | 12.2 | 11.4 | 2.2 | 15 | 6.5 | 6.2 | 6.1 | 6.0 | 5.8 | 5.3 | 1.2 |
| 6 | 11.5 | 11.3 | 11.5 | 10.4 | 11.8 | 10.9 | 1.4 | 16 | 6.9 | 7.1 | 6.9 | 7.4 | 6.9 | 7.0 | 0.5 |
| 7 | 11.1 | 13.6 | 13.3 | 13.2 | 12.6 | 13.8 | 2.7 | | | | | | | | |
| 8 | 9.3 | 10.7 | 10.8 | 9.9 | 9.2 | 9.7 | 1.6 | | Average | | | | | | 0.88 |
| 9 | 12.3 | 14.1 | 14.4 | 13.4 | 13.2 | 13.8 | 2.1 | | | | | | | | |
| 10 | 9.6 | 9.0 | 8.5 | 9.6 | 9.0 | 9.0 | 1.1 | | | | | | | | |
| | Average | | | | | | 1.69 | | | | | | | | |
| | Grams of Hemoglobin per Cell × $10^{-11}$ | | | | | | Maximum | | Grams of Hemoglobin per Cell × $10^{-11}$ | | | | | | Maximum |
| Case | 8 a. m. | 10 a. m. | 12 m. | 2 p. m. | 4 p. m. | 6 p. m. | Variation | Case | 8 a. m. | 10 a. m. | 12 m. | 2 p. m. | 4 p. m. | 6 p. m. | Variation |
| 1 | 4.9 | 4.3 | 4.5 | 5.0 | 4.1 | 4.4 | 1.0 | 11 | 1.4 | 1.3 | 1.5 | 1.5 | 1.4 | 1.3 | 0.2 |
| 2 | 4.6 | 4.3 | 5.1 | 4.9 | 5.0 | 4.4 | 0.8 | 12 | 1.9 | 1.9 | 2.0 | 2.1 | 2.1 | 2.0 | 0.2 |
| 3 | 4.1 | 4.1 | 4.0 | 4.0 | 4.4 | 4.1 | 0.4 | 13 | 2.7 | 2.7 | 2.4 | 2.7 | 2.7 | 2.7 | 0.3 |
| 4 | 5.6 | 5.1 | 4.8 | 5.1 | 5.2 | 5.3 | 0.8 | 14 | 1.6 | 1.7 | 1.7 | 1.7 | 1.8 | 1.7 | 0.2 |
| 5 | 4.4 | 4.4 | 4.3 | 4.7 | 5.2 | 4.8 | 0.9 | 15 | 1.6 | 1.6 | 1.6 | 1.6 | 1.5 | 1.5 | 0.1 |
| 6 | 4.6 | 4.4 | 4.5 | 4.2 | 4.5 | 4.5 | 0.4 | 16 | 1.8 | 1.9 | 2.1 | 2.4 | 2.2 | 2.3 | 0.6 |
| 7 | 4.6 | 5.0 | 5.0 | 4.9 | 4.8 | 5.6 | 1.0 | | | | | | | | |
| 8 | 3.5 | 4.1 | 3.6 | 3.5 | 3.6 | 3.3 | 0.8 | | Average | | | | | | 0.27 |
| 9 | 4.8 | 5.3 | 5.7 | 5.2 | 4.8 | 5.2 | 0.9 | | | | | | | | |
| 10 | 3.0 | 2.6 | 2.7 | 2.6 | 2.7 | 2.6 | 0.4 | | | | | | | | |
| | Average | | | | | | 0.74 | | | | | | | | |

Experiments on cases of anemia showing hourly variations in the number, size and hemoglobin content of the red cell furnish data which, although not convincing in all instances from the point of view of this hypothesis, do support the latter in the same proportion of cases. In support of this conclusion, the graphic evidence in the accompanying charts is presented. In the main, Cases 15, 8, 12, 14, 10 and 1 favor; Cases 9, 7, 2, 6, 5, 3, 4, 11, 13 and 14 present evidence to the contrary. In order to demonstrate that this is so, it is only necessary to consider the curves representing the average size of the red cell and the percentage of hemoglobin. When the average size of the cell increases, it means that per unit volume there is less cell surface, and, if hemoglobin has a surface distribution, then when the curve representing

the average size of the red cell rises, that representing the percentage of hemoglobin should fall. That this does occur is shown by the first group of cases. The others show a reverse condition. It may be seen at a glance that, in the case of the second group, the curve designating the percentage of hemoglobin is roughly parallel to that representing the average size of the cell, and the latter is inversely proportional to the cell surface per unit volume.

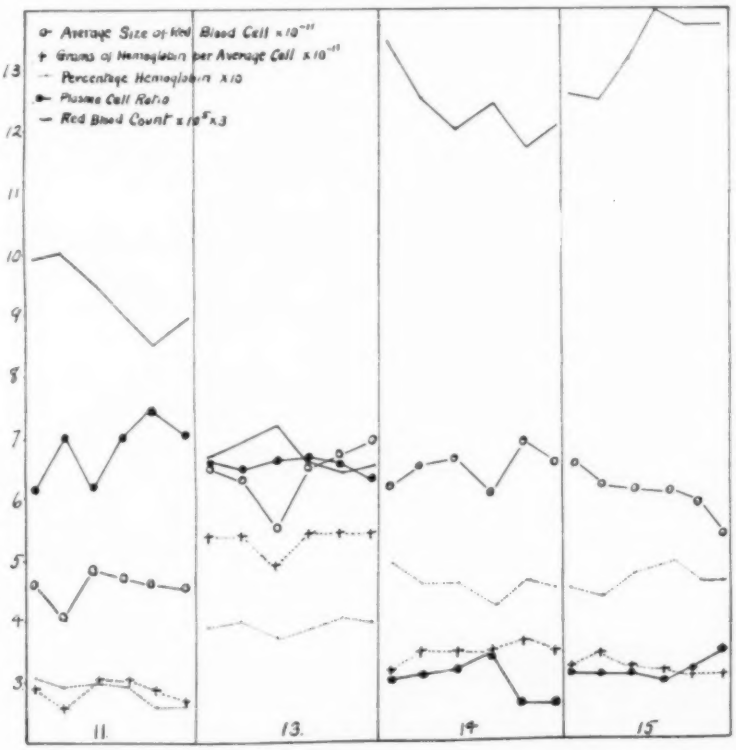


Chart 4

## CONCLUSIONS

Hourly variations in the percentage of hemoglobin of the blood, which have been shown to occur in normal persons, do take place in anemia, though to a less extent. Hourly variations in the average size of the erythrocyte may be considerable. A definite relation between the percentage of hemoglobin and the cell surface seems to exist in a certain percentage of the cases.

# THE ELECTROCARDIOGRAM AND BLOOD PRESSURE DURING SURGICAL OPERATION AND CONVALESCENCE

## OBSERVATIONS ON THIRTY PATIENTS *

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NEW HAVEN, CONN.

It is apparently the belief of many surgeons and internists that postoperative complications may be lessened or prevented by the preoperative administration of digitalis, even to patients whose hearts are apparently normal.[1] The present investigation was undertaken in an attempt to determine the influence of digitalis on certain objective factors during and after surgical operation, but, as no similar study has been found in previous reports, it seemed necessary to establish first a control group of nondigitalized patients for subsequent comparison with a similar group of patients who had received digitalis. It is the nondigitalized group which forms the basis for the present report; the influence of digitalis is discussed in the following paper.

### METHOD OF STUDY

It has been our purpose to secure records of the blood pressure and electrocardiograms at frequent intervals during the induction of anesthesia and operation, and records of the blood pressure and clinical course during ten or more days of convalescence. There was no selection of patients on the basis of age, sex or cardiac condition, but an effort was made to secure a wide variety of the more serious operative conditions. The preoperative blood pressure was obtained, in most instances, at least twenty-four hours before operation, and patients with auricular fibrillation or hypertension had several readings taken, in order that the usual range of variation might be determined. The subjects were connected with the electrocardiograph and the blood pressure manometer in the anesthesia room before the anesthetic was started, and preliminary records were secured. One observer remained in the operating room from the beginning of anesthesia until the end of the operation to determine the blood pressures and to signal for electrocardiograms. He was seated within several feet of the patient so that the effect of trauma, traction on the viscera, hemorrhage and depth of

* From the Department of Internal Medicine, Yale University School of Medicine, and the New Haven Hospital.

1. References may be found in Marvin, H. M.; Pastor, R. B., and Carmichael, M.: Effect of Routine Preoperative Digitalization on the Electrocardiogram and Blood Pressure During Surgical Operation and Convalescence, Arch. Int. Med., this issue.

anesthesia could be noted. There was constant connection with the electrocardiographic laboratory through a special telephone and electric buzzer.

As a general rule, the blood pressure was determined every two and a half minutes, and at every second or third determination an electrocardiogram was taken simultaneously in response to a signal. Observations were made more frequently if unusual circumstances arose, and it was always possible to determine whether changes in blood pressure or heart rate were dependent on a new cardiac mechanism or some other factor. Most electrocardiagrams were of Lead II, but all three leads were taken at the beginning, at intervals during, and at the conclusion of, the operation. About 750 records were secured from the thirty patients who were studied.

Postoperative observations began approximately six hours after the conclusion of the operation, and were made three times a day for the first four days, twice a day for the next four days, then once a day until the patient was discharged or convalescence was well established. These observations included the determination of the systolic and diastolic pressures and careful notes as to the occurrence of nausea and vomiting, the rapidity of convalescence, the presence of complications, and the general clinical condition of the patient. The total number of blood pressure readings, including those secured during operations, was more than 2,400; an average of about eighty for each patient. Electrocardiograms were taken during convalescence only when there was some indication for them.

The sphygmomanometers used were of the standard mercury type; the same two instruments were used throughout the study. All determinations, as well as all clinical notes, were made by the authors, whose readings corresponded accurately. The fourth phase was taken as the diastolic pressure in all cases, and in patients with auricular fibrillation the maximal beats were taken as the systolic pressure. Thirty patients were studied.

## BLOOD PRESSURE DURING OPERATION

It is notoriously difficult to draw satisfactory conclusions as to the cause of moderate changes in blood pressure because of the multiplicity of factors involved, many of them but little understood. It has seemed advisable to classify our results only in a general way, and the cases have been arbitrarily arranged in four groups: (*a*) Those in whom the blood pressure rose during the induction of anesthesia and remained above the preoperative level during the entire operation; (*b*) those in whom the blood pressure, after a preliminary rise, fell and remained below the preoperative level throughout the operation; (*c*) those in whom the pressure fluctuated within wide limits, both above and below the preoperative blood pressure, and (*d*) those in whom the blood pressure,

during operation, remained at the preoperative level. This same classification has been applied to the postoperative blood pressures obtained after the first day, but those obtained at the end of six, eighteen, twenty-four and thirty hours after operation have been listed separately for the sake of more accurate comparison with our digitalized patients and with the observations of other authors.

It should be noted that, in this classification, blood pressures have been considered "above" or "below" the preoperative pressure only when the difference between them was greater than 4 mm. of mercury. If the two pressures differed by 4 mm. or less, it was considered that there had been no change. We believe this difference to lie within the limit of error of observation.

Almost without exception, there was a rise in both systolic and diastolic pressures during the induction of general anesthesia; this has been noted so often as to need no discussion. If this elevation proved transient, as in many cases, it was disregarded for purposes of classification. It also was noted that the blood pressure in many patients rose and fell directly with the depth of anesthesia, and that there was almost always less fluctuation in pressure if the anesthesia was fairly deep. The operative procedure itself seemed to have no influence on the blood pressure unless it involved obstruction to the respiration (operations on the throat and jaw) or was continued for four or five hours. It was possible often to confirm the statement of Miller [2] that "the most severe surgical manipulations may regularly be performed without marked change in either the blood pressure or the pulse rate." In a few patients, posture seemed to be responsible for changes in pressure; a change to the Trendelenburg position was occasionally followed promptly by a fall in blood pressure which was corrected when the table was again lowered; in a very few, a steadily falling blood pressure was apparently stopped from further depression by lowering the head of the table.

Apart from these general considerations, it is of interest to observe the relation of the operative to the preoperative blood pressures in the present series. One patient has been omitted because, at the time of admission to the accident room, the systolic pressure was so low as to be unobtainable. Of the remaining twenty-nine, the blood pressure during operation was constantly above the preoperative level in thirteen, constantly below in thirteen, fluctuating above and below in two, and the same in one. The distribution of these groups with regard to the type of anesthesia is shown in Table 1.

Five patients developed "surgical shock" during operation. In all of them, the steady fall in blood pressure was the first indication of danger,

2. Miller, A. H.: Blood Pressure Guides During Anesthesia and Operation, Am. J. Surg. (Anesthesia Supplement) **35**:34 (April) 1921.

and in two of them it preceded other evidences by a considerable period of time.

### BLOOD PRESSURE DURING THE THIRTY HOURS AFTER OPERATION

The figures obtained during the first thirty hours following operation are perhaps of the greatest interest. Geist and Somberg [3] and Geist and Goldberger [4] have laid great emphasis on a postoperative fall in blood pressure as an apparent cause of postoperative complications. Their observations in thirty-nine patients showed an average fall in blood pressure of 14 mm. at six, twelve and twenty-four hours after operation. They quote Polak [5] as also having shown an average fall of 14.2 mm. in the blood pressure after operation, but Polak's readings were made only one hour after the conclusion of the operation, and apply only to etherized patients. Lehrnbecher [6] feels that the blood pressures immediately after operation is of little importance, but that those obtained in the evening and on the morning following are of considerable aid in prognosis.

TABLE 1.—*Blood Pressures During Operation in Twenty-Nine Cases* *

| Anesthesia | Above | Below | Same | Fluctuating |
|---|---|---|---|---|
| Gas-oxygen (nitrous oxid) | 6 | 8 | 1 | 1 |
| Ether | 2 | 4 | .. | .. |
| Procain | 4 | 1 | .. | 1 |
| None | 1 | .. | .. | .. |

* As compared with the preoperative blood pressure.

The figures obtained in the present study fail to show a fall in the systolic blood pressure after operation in the majority of cases. The records of four patients, Cases 16, 25, 28 and 30, were not included in the following analysis; one of them because the preoperative blood pressure could not be obtained, and the other three because they were in a condition of shock at the end of operation, and the blood pressure rose slowly throughout the following day. Of the remaining twenty-six, there were only eight (31 per cent.) whose blood pressure at the end of six hours was lower than the preoperative pressure, while at the end

3. Geist, S. H., and Somberg, J. S.: Preoperative Digitalization: A Method to Reduce Postoperative Complications, Am. J. Obst. & Gynec. **4**:135 (Aug.) 1922.

4. Geist, S. H., and Goldberger, M. A.: Effect of Preoperative Digitalization in Reducing Postoperative Complications, Ann. Surg. **78**:693 (Dec.) 1923.

5. Polak, J. O.: A Clinical Study in Preoperative and Postoperative Blood Pressures and Their Relation to Renal Function and in Shock and Hemorrhage, Am. J. Obst. **80**:113 (Aug.) 1919.

6. Lehrnbecher, A.: Die theoretische Grundlage und praktische Anwendung der Blutdruckmessung bei chirurgischen Eingriffen, Beitr. z. klin. Chir. **127**: 291, 1922.

of eighteen hours the pressure had returned to or above the preoperative level in all except five (19 per cent.).

If the figures are analyzed with regard to the relation of the postoperative pulse pressure to the preoperative pulse pressure, the results are slightly different but they point to a similar conclusion: that the great majority of the patients during the thirty hours following operation have pulse pressures as great as, or greater than, before operation. The number of patients who showed changes in the systolic and pulse pressures at intervals after operation is shown in Table 2.

The eight patients who showed a transient fall in blood pressure after operation (Cases 3, 12, 13, 20, 21, 22, 23 and 24) all had uneventful recoveries

### GENERAL POSTOPERATIVE BLOOD PRESSURES

Under the term "general postoperative blood pressures," we have classified all those obtained after the first thirty hours following operation; the readings begin, therefore, with the morning of the second day. The figures obtained for each patient have been charted and the relation

TABLE 2.—*Blood Pressures During the Thirty Hours After Operation* *

| | Systolic Pressure | | | Pulse Pressure | | |
|---|---|---|---|---|---|---|
| | Above | Below | Same | Above | Below | Same |
| 6 hours after operation | 11 | 8 | 7 | 8 | 9 | 9 |
| 18 hours after operation | 14 | 5 | 7 | 10 | 8 | 8 |
| 24 hours after operation | 13 | 6 | 6 | 6 | 8 | 11 |
| 30 hours after operation | 11 | 8 | 10 | 7 | 4 | 13 |

* As compared with the preoperative systolic and pulse pressures.

of the curve to the preoperative level established. Sharp deviations of the blood pressure above or below the general level which were noted at only one observation have been disregarded for purposes of classification. Two patients died within three days after operation; of the remaining twenty-eight whose courses were followed for periods of from eight to twenty days after operation, the blood pressure was constantly above the preoperative level in nine, constantly below in twelve, and the same in seven.

### COMPLICATIONS

There were postoperative complications in four patients, all of whom developed bronchopneumonia; one of them had erysipelas also. Signs of pneumonia were detected in two patients on the second day after operation, in one on the third, and in one on the sixth. In two instances (Cases 25 and 26), it followed operations on the jaw and pharynx which were attended by severe trauma and undoubted inhalation of blood and mucus; in the other two (Cases 7 and 10), it occurred after upper abdominal operations in patients whose anesthetization was marked by unusual struggling and vomiting. For the purpose of this study, it is

TABLE 3.—*Blood Pressures in Nondigitalized Cases**

| Case | Age | Operation | Length of Operation | | Anesthesia | Preoperative Blood Pressure | | General Operative Blood Pressure | Postoperative Blood Pressure | | | | | | | | General Postoperative Blood Pressure | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 6 Hr. | | 18 Hr. | | 24 Hr. | | 30 Hr. | | | |
| | | | Hr. | Min. | | Systolic | Diastolic | | Systolic | Diastolic | Systolic | Diastolic | Systolic | Diastolic | Systolic | Diastolic | | |
| 1 | 54 | Cervical sympathectomy | 2 | 10 | Local | 130 | 78 | Above | 136 | 82 | 140 | 80 | ... | ... | 136 | 76 | Above | Arteriosclerotic heart disease; anginal failure |
| 2 | 48 | Cervical sympathectomy | 2 | 16 | Local, gas-oxygen | 126 | 60 | Fluctuating | 134 | 60 | 132 | 58 | 132 | 60 | ... | ... | Above | Syphilitic heart disease; aortic insufficiency and anginal failure |
| 3 | 38 | Exploration | 1 | 43 | Gas-oxygen | 110 | 68 | Above | 100 | 70 | 110 | 84 | 100 | 82 | 112 | 90 | Below | Gumma of liver; excellent recovery |
| 4 | 63 | Colostomy | 1 | 40 | Gas-oxygen | 98 | 48 | Above | 98 | 58 | 110 | 68 | 112 | 70 | 118 | 70 | Fluctuating | Carcinoma, rectum |
| 5 | 42 | Resection, sigmoid | 2 | .. | Gas-oxygen | 112 | 82 | Above | 154 | 90 | 154 | 92 | 156 | 94 | 152 | 94 | Fluctuating | |
| 6 | 40 | Resection, colon | .. | 45 | None | 152 | 90 | Above | 168 | 92 | 168 | 94 | 162 | 96 | 152 | 88 | Below | |
| 7 | 38 | Perforated gastric ulcer | 1 | 22 | Ether | 110 | 70 | Below | 120 | 80 | 120 | 80 | 122 | 80 | 126 | 82 | Fluctuating | Acute delirium; bronchopneumonia |
| 8 | 38 | Gastro-enterostomy | 2 | 22 | Local; gas-oxygen | 130 | 58 | Above | 138 | 36 | 134 | 40 | 132 | 38 | 136 | 42 | Below | Syphilitic heart disease; aortic insufficiency |
| 9 | 67 | Resection, stomach | 3 | 5 | Gas-oxygen | 94 | 70 | Fluctuating | 128 | 70 | 134 | 70 | 128 | 72 | 124 | 68 | Above | |
| 10 | 28 | Cholecystectomy | 1 | 30 | Gas-oxygen | 126 | 78 | Above | 130 | 86 | 124 | 95 | 124 | 88 | 122 | 72 | Fluctuating | Postoperative bronchopneumonia and erysipelas |
| 11 | 26 | Cholecystectomy | 2 | .. | Gas-oxygen | 118 | 70 | Below | 145 | 100 | 142 | 88 | 142 | 94 | 150 | 84 | Fluctuating | |
| 12 | 16 | Nephrectomy | 2 | 30 | Gas-oxygen | 106 | 92 | Steady fall | 146 | 92 | 172 | 92 | 172 | 94 | 168 | 98 | Fluctuating | Excellent recovery |
| 13 | 23 | Ligation, superior thyroid artery | .. | 30 | Gas-oxygen | 100 | 84 | Below | 154 | 82 | 146 | 76 | 148 | 78 | 148 | 74 | Below | Thyrotoxicosis; excellent recovery |
| 14 | 23 | Thyroidectomy | .. | 50 | Local | 160 | 80 | Above | 180 | 96 | 184 | 98 | 180 | 98 | 170 | 94 | Below | Thyrotoxicosis |
| 15 | 26 | Cardiolysis | 1 | 46 | Local | 98 | 52 | Below | 100 | 60 | 100 | 58 | 102 | 60 | 102 | 62 | Above | Rheumatic heart disease; adhesive pericarditis |
| 16 | 14 | Pericardiotomy | 1 | 15 | Ether | 45 | ? | Not taken | 114 | 70 | 110 | 60 | 110 | 70 | 104 | 70 | Above | Stab wound of heart |
| 17 | 74 | Amputation of leg | 1 | 15 | Gas-oxygen | 150 | 82 | Above | 130 | 78 | 128 | 76 | 138 | 74 | 136 | 82 | Above | Arteriosclerotic heart disease; auricular fibrillation |
| 18 | 9 | Open reduction, tibia | 1 | 55 | Ether | 110 | 80 | Above | 108 | 70 | 110 | 80 | 108 | 78 | 108 | 78 | Below | |
| 19 | 61 | Cystotomy | .. | 52 | Gas-oxygen | 120 | 72 | Above | 118 | 72 | 120 | 70 | 118 | 72 | 118 | 72 | Below | |
| 20 | 56 | Prostatectomy | 1 | 48 | Gas-oxygen | 145 | 80 | Below | 140 | 86 | 130 | 74 | 134 | 78 | 144 | 78 | Fluctuating | Excellent recovery |
| 21 | 22 | Hysterectomy | 1 | 15 | Gas-oxygen | 118 | 66 | Below | 92 | 58 | 140 | 90 | 126 | 72 | 130 | 82 | Above | Shock from hemorrhage; excellent recovery |
| 22 | 47 | Panhysterectomy | 2 | 45 | Gas-oxygen | 154 | 80 | Below | 112 | 84 | 138 | 94 | 142 | 92 | ... | ... | Below | Excellent recovery |
| 23 | 37 | Cesarean section | 1 | 30 | Gas-oxygen | 152 | 90 | Below | 128 | 84 | 120 | 82 | 122 | 80 | 136 | 74 | Below | Rheumatic heart disease; excellent recovery |
| 24 | 23 | Hysterectomy | 2 | .. | Gas-oxygen | 140 | 82 | Below | 132 | 80 | 130 | 82 | 128 | 84 | 128 | 78 | Below | Excellent recovery |
| 25 | 56 | Carcinoma, jaw and neck | 3 | 48 | Ether | 98 | 60 | Steady fall | * | | 90 | 50 | 92 | 60 | 90 | 54 | Below | Marked trauma; died of pneumonia |
| 26 | 74 | Carcinoma, jaw | 3 | 15 | Local, chloroform | 118 | 68 | Above | 142 | 70 | 155 | 78 | 160 | 76 | 156 | 80 | Above | Marked trauma; died of pneumonia |
| 27 | 39 | Bilateral herniotomy | 2 | 10 | Gas-oxygen | 124 | 84 | Same | 122 | 82 | 126 | 88 | 126 | 86 | 126 | 82 | Above | |
| 28 | 56 | Bilateral breast cancer | 3 | .. | Ether | 236 | 110 | Steady fall | 142 | 70 | 216 | 132 | 204 | 112 | 202 | 114 | Below | Shock |
| 29 | 45 | Decompression | 2 | 12 | Ether | 106 | 74 | Above | 124 | 90 | 132 | 90 | 124 | 90 | 134 | 92 | Above | |
| 30 | 45 | Glioma of brain | 5 | .. | Ether | 120 | 88 | Steady fall | 82 | 58 | 110 | 78 | 133 | 84 | 110 | 76 | Below | Shock |

* Blood pressure too low to be obtained.

important to observe that, in three of these four patients, the blood pressure, during the thirty hours following operation, was at or above the preoperative level, while in the fourth, a patient who left the table in profound shock, the pressure rose subsequently almost to the level noted before operation. Our findings, in this respect, differ from those of Geist and his collaborators, who observed pulmonary complications only in those patients who had shown a postoperative fall in blood pressure.

A summary of the thirty cases, with blood pressure changes during and after operation, is given in Table 3.

### ELECTROCARDIOGRAPHIC STUDIES

We have found only three references to the study of electrocardiograms taken during or immediately following surgical operations. In 1918, Heard and Strauss [7] reported an instance of atrioventricular rhythm occurring in a patient still under the influence of ether following operation. Levine [8] later reported two cases of paroxysmal auricular tachycardia detected by means of electrocardiograms in patients who had been operated on and were still under ether. The only comprehensive study appears to be that of Lennox, Graves and Levine,[9] who secured frequent records from fifty patients during operation. These observers state in conclusion:

> About one half of the cases showed some abnormality of the mechanism of the heart beat not present in preliminary tracings, the most prominent being paroxysmal auricular tachycardia, premature beats, and marked displacement of the cardiac pacemaker. The observed abnormalities of the heart beat were transient and unassociated with clinical signs of embarrassed circulation. In the main, they were of physiological rather than of clinical significance.

There is a striking similarity between the findings in our patients and those of the authors just quoted, as regards the percentage and character of electrocardiographic changes. Fifteen of our thirty cases (50 per cent.) showed some definite abnormality during operation which was not present in preliminary tracings. Sino-auricular, or physiologic tachycardia has not been regarded as abnormal, although several patients maintained a rate distinctly above normal throughout operation. The observed changes are discussed below.

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7. Heard, J. D., and Strauss, A. E.: An Electrocardiographic Study of Two Cases of Nodal Rhythm Exhibiting R-P Intervals, Am. J. M. Sc. **75**:238, 1918.

8. Levine: S. A.: Acute Cardiac Upsets Occurring During or Following Surgical Operations, J. A. M. A. **75**:796 (Sept. 18) 1920.

9. Lennox, W. G.; Graves, R. C., and Levine, S. A.: An Electrocardiographic Study of Fifty Patients During Operation, Arch. Int. Med. **30**:57 (July) 1922.

### SINUS ARRHYTHMIA AND BRADYCARDIA

Only one patient exhibited sinus arrhythmia; she was likewise the only one in whom bradycardia was noted almost constantly. She was 54 years of age, and was considered to have definite arteriosclerotic heart disease with angina pectoris. The operation was resection of the left cervical sympathetic nerve, with removal of the superior cervical ganglion; it was performed under procain anesthesia. The vagus and sympathetic trunks lay unusually near each other in this patient, and there was considerable vagal stimulation, some of it deliberate. It is quite possible that the arrhythmia may have been a vagal effect.

### PREMATURE BEATS

Premature beats were registered in seven patients, none of whom had shown such beats in the preliminary tracings or during physical

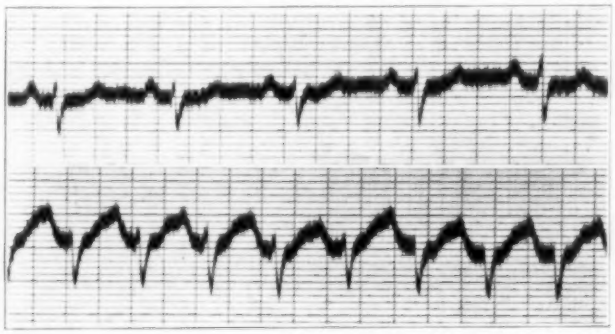

Fig. 1. (Case 26).—Lead II: above, normal mechanism with rate of 80 a minute; below, paroxysmal tachycardia with rate of 142 a minute. In this and the following electrocardiograms, distances between abscissas represent $10^{-4}$ volts, and time is in one-fifth seconds.

examination. In six of them, the ectopic beats arose in the ventricles; in the seventh, the origin was in the junctional tissues. Two of these patients were elderly, with peripheral arteriosclerosis and evidences of arteriosclerotic heart disease; one of them had also auricular fibrillation. No abnormality of the cardiovascular system was detected in the remaining five. The premature beats occurred with greater frequency during the induction of anesthesia or the early part of the operation, but in three patients they were recorded at intervals throughout the operation.

### INCREASE IN SIZE OF T WAVE

In one patient there was a remarkable increase in the amplitude of the T wave in Lead II during the early part of the operation. This

wave, which was upright at all times, became approximately twice as high as normal without alterations in any other deflections. It returned to its previous size before the operation had been finished.

### PAROXYSMAL TACHYCARDIA

There were two instances of paroxysmal tachycardia; in one patient the arrhythmia was noted in only one record, but, in the other, the paroxysms occurred throughout the period of operation. They were unaccompanied by signs of circulatory embarrassment. It is of some interest to note that both these patients received ether through a nasal tube introduced into the pharynx. The electrocardiograms of Case 26 are shown in Figure 1.

A third patient during operation showed short runs of ventricular premature beats which might strictly be interpreted as paroxysms of ventricular tachycardia, but these occurred at a time when the heart was being handled by the surgeon, who was removing blood from the peri-

Fig. 2 (Case 16).—Lead II: series of ectopic ventricular beats which occurred during handling of heart; first complex in record is a normal one with the T wave slightly distorted.

cardial sac. While the mechanical stimulation of the heart was doubtless responsible for the series of ectopic beats, illustrated in Figure 2, isolated premature beats were registered before the pericardium was opened, so the case has been listed with that group.

### DISPLACEMENT OF THE PACEMAKER

The most frequent abnormality in our records was a conspicuous change in the P wave, which occurred in seven patients (23 per cent.). This is in accord with the observations of Lennox, Graves and Levine, who found such changes in 30 per cent. of their patients during operation. In the present study, the changes were of only two types: (1) a complete disappearance of the P wave, which apparently became buried in the Q-R-S complex, and (2) the appearance of an inverted P wave following the Q-R-S complex. Seven patients showed the first type; one of them in addition showed the second.

It seems fairly clear that a majority, if not all, of such changes represent a displacement of the pacemaker downward, usually into the auriculoventricular node, but Levine [9] has clearly pointed out the difficulties which lie in the way of that assumption, and has emphasized the lack of agreement as to the criteria by which auriculoventricular rhythm is to be diagnosed from electrocardiograms. We have followed his

Fig. 3 (Case 21).—Lead II: above, normal mechanism; below, displacement of pacemaker. Absence of P waves in lower record should be noted.

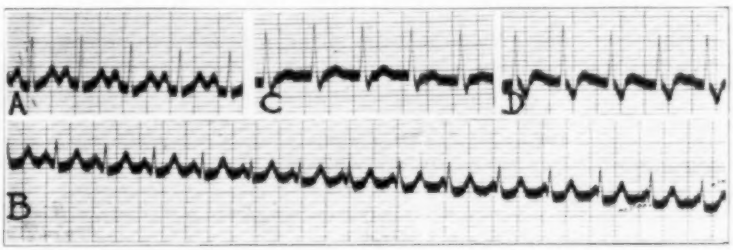


Fig. 4.—*A*, Lead II, normal mechanism; *B*, Lead III, taken a few seconds later, showing the gradual merging of the P Wave with the Q-R-S group; *C* and *D*, Lead II taken at intervals of about three minutes, showing different types of displacement of the pacemaker. *A* and *B* were made before induction of anesthesia, *C* and *D* during induction.

example in referring to this group as simply "displacement" of the pacemaker. Figure 3 shows the change which characterized the record of six patients; they were so uniform that only one has been reproduced. In Figure 4, there is clearly shown the two different mechanisms which controlled the heart in Case 23. The portion of this figure labelled *B* was taken a few seconds after *A*, and shows the gradual shortening of the P-R interval until the P wave is lost in the Q-R-S group. About

three minutes elapsed before *C* was taken, and an equal interval between *C* and *D*, both of which are of Lead II. In the former of these, no P waves are visible; in the latter, the inverted waves immediately following the Q-R-S complex are thought to be retrograde auricular waves. Every tracing secured during operation from this patient showed the mechanism illustrated in *C* or *D*, but an electrocardiogram taken several days later showed normal mechanism.

It should be pointed out that the displacement of the pacemaker in this case occurred before anesthesia had been started; the first two records in Figure 4 were taken while the patient was in the anesthesia room awaiting the arrival of the surgeon. This was the only instance in which a transient abnormality was noted in the preliminary tracings, but it affords further evidence of the ease with which the pacemaker may be disturbed. The presence of rheumatic heart disease with mitral stenosis and insufficiency may have had some bearing on the early appearance and long duration of the arrhythmia in this case.

No one factor has been discovered which might be of importance in causing this change in the electrocardiogram. Of the patients manifesting it, about half were below the age of 40, and half above; the sexes were almost equally represented; the changes occurred with both ether and nitrous oxid-oxygen anesthesia, and the operations involved such diverse parts of the body as the uterus, stomach, jaw and brain. The length of anesthesia varied from one hour and fifteen minutes to slightly more than five hours, but in no instance did a displacement of the pacemaker occur during the latter part of a long operation.

### RELATION OF ELECTROCARDIOGRAPHIC CHANGES TO BLOOD PRESSURE

The determination of the blood pressure simultaneously with the registration of the electrocardiogram has afforded an opportunity to study a possible relation between disturbed cardiac mechanism and changes in blood pressure. We have failed to establish any such relation. In several cases, there seemed to be a simultaneous change in blood pressure and electrocardiogram, but, in every instance, the change in pressure was maintained, while the electrocardiographic change was transient. In many cases, the arrhythmia recurred, and in none was the blood pressure the same during the successive episodes.

A summary of the changes in blood pressure associated with variations in the electrocardiogram is given in Table 4. When two blood pressures are given, they represent the readings taken simultaneously with the first and last electrocardiograms that displayed the abnormal mechanism.

TABLE 4.—*Electrocardiograms and Blood Pressures*

| Case | Age | Cardiac Diagnosis | Preoperative Blood Pressure | | Abnormalities in Electrocardiograms | Simultaneous Blood Pressure | | Remarks |
|---|---|---|---|---|---|---|---|---|
| | | | Systolic | Diastolic | | Systolic | Diastolic | |
| 1 | 54 | Arteriosclerotic heart disease with anginal failure | 130 | 78 | Sinus arrhythmia and bradycardia | 156 | 84 | Pulse slowed when working near vagus nerve |
| | | | | | Junctional premature beats | 160 | 84 | |
| 3 | 38 | Normal | 110 | 68 | Displacement of pacemaker | 126 | 80 | |
| | | | | | Ventricular premature beats | 140 | 88 | |
| 4 | 63 | Normal | 98 | 48 | Ventricular premature beats | 160 | 84 | |
| | | | | | | 134 | 60 | |
| 8 | 38 | Syphilitic heart disease; aortic insufficiency | 130 | 38 | Increase in size of T wave in Lead II | 142 | 48 | |
| 10 | 28 | Normal | 126 | 78 | Displacement of pacemaker | 142 | 86 | |
| 16 | 14 | Stab wound of heart | 45 | ? | Ectopic ventricular beats isolated and in short paroxysms | Unobtainable | | Heart handled during paroxysms |
| 17 | 74 | Arteriosclerotic heart disease; auricular fibrillation | 120 | 82 | Ectopic ventricular beats | 140 | 96 | |
| 20 | 56 | Normal | 145 | 80 | Displacement of pacemaker | 170 | 106 | |
| 21 | 22 | Normal | 118 | 68 | Displacement of pacemaker | 100 | 72 | Shock from hemorrhage |
| | | | | | | 110 | 64 | |
| | | | | | | 110 | 50 | |
| 22 | 47 | Normal | 154 | 80 | Ventricular premature beats | 188 | 104 | |
| 23 | 37 | Rheumatic heart disease | 152 | 90 | Displacement of pacemaker | 152 | 90 | Onset of auriculoventricular rhythm before anesthesia |
| | | | | | | 128 | 84 | |
| 25 | 56 | Normal | 98 | 60 | Displacement of pacemaker | 134 | 74 | Marked trauma; shock |
| 26 | 74 | Normal | 118 | 68 | Paroxysmal tachycardia | 178 | 90 | |
| | | | | | Ventricular premature beats | 130 | 78 | |
| 30 | 45 | Normal | 120 | 88 | Displacement of pacemaker | 104 | 78 | Shock |
| | | | | | | 144 | 88 | |
| | | | | | Paroxysmal junctional tachycardia | 180 | 112 | |

### RELATION OF CHANGES TO CARDIAC PATHOLOGY

Six patients in this series had definite signs of heart disease. In two of them, the etiology was rheumatic infection, in two it was syphilitic, and in two the changes were apparently arteriosclerotic in origin. Anginal heart failure was present in two patients, but none showed evidences of congestive failure. From the standpoint of blood pressure changes and clinical course, these patients withstood operation as well as any normal individual. Four of them, it is true, showed electrocardiographic changes during operation, but in one they were probably due to direct vagal stimulation. If that minor change be disregarded, the percentage of electrocardiographic changes in the cardiac patients becomes practically the same as that in normal persons.

### SIGNIFICANCE OF ELECTROCARDIOGRAPHIC CHANGES

Of the four patients who developed surgical shock, three showed a displacement of the pacemaker in electrocardiograms. But in these three,

the electrocardiographic change was transient and had disappeared long before signs of shock appeared; in all of them, moreover, there was an adequate apparent cause for the shock quite apart from a minor change in the cardiac mechanism. In one, there was profuse hemorrhage; in another, the operation on the brain lasted five hours, and in the third there was prolonged and extensive cauterization of the jaw, neck and pharynx in a patient whose anesthetization was difficult and very unsatisfactory. In none of the other patients was there any evidence that the abnormalities in the electrocardiogram had a clinical significance, either at the time of their occurrence or afterward.

## COMMENT

On many occasions during this study, patients had the blood pressure determined as often as once a minute for considerable periods of time, and almost without exception readings were taken every two and a half minutes. These frequent observations have shown that the pressure may fluctuate within wide limits above and below its usual level without other apparent change in the circulation or general condition of the patient. In this study, the only change which has seemed of significance has been the steady and conspicuous fall in pressure which accompanied the appearance of surgical shock. Rises in pressure as great as 100 mm. above the preoperative level have been unassociated with signs of circulatory disturbance, and significant lowering (amounting to half the systolic pressure in hypertensive individuals) has likewise been without clinical manifestations unless it reached levels of from 80 to 90 mm. of mercury. The rarity or frequency of fluctuations in the blood pressure level has had no apparent significance, and it appears certain that the behavior of the blood pressure during operation has afforded no indication of its subsequent behavior or of the clinical course of the patient. The changes in the electrocardiogram during operation were largely of a minor character, and had no demonstrable influence on the circulation so far as could be determined by blood pressure readings and clinical observations. We are in complete agreement with those previous observers who considered them of physiologic interest rather than of clinical importance.

With regard to determinations of the blood pressure after operation, it may be said that they have been of no value whatver in estimating the danger of postoperative complications or the type and duration of convalescence. This statement does not apply to those patients who left the operating table in shock; in them, frequent observation of the blood pressure seemed essential. Attention has already been directed to the fact that very few of the patients in the present series showed a postoperative fall in pressure, and that convalescence in those few was uneventful. A careful comparison of clinical notes and blood pressure

records obtained during the ten days following operation fails to disclose any constant relation between them, except that during the later days of convalescence many patients had a pressure lower than before operation. That this moderate fall may be attributed largely to prolonged rest in bed is indicated by similar findings in many other medical and surgical patients who were not subjected to anesthesia and operation.

The four patients who developed postoperative complications did not have a postoperative lowering of the blood pressure, or any other changes which were distinctive.

## SUMMARY

The blood pressure has been determined, and electrocardiograms taken, at frequent intervals during anesthesia and operation in thirty patients. Blood pressures were determined and clinical notes made at regular intervals each day for ten or more days of convalescence.

The blood pressure during operation varied considerably; it was constantly above and constantly below the preoperative level in an equal number of cases. Changes in pressure in either direction seemed to be without clinical significance except when there was extreme lowering of the systolic and pulse pressures in cases of shock.

The majority of patients, in this series, failed to show the postoperative fall in systolic and pulse pressures reported by some observers and assumed by many. Of the few patients who did show such a fall, none developed postoperative complications or had a protracted convalescence.

Blood pressures during the first thirty hours after operation, as well as those taken during the following ten days, were found to be of no value in estimating the likelihood of complications or the rapidity of convalescence.

Electrocardiograms taken during operation showed definite changes, not present in preliminary tracings, in about half the cases. The chief of these were: premature beats, paroxysmal tachycardia and disturbances of the pacemaker. They were not associated with demonstrable changes in the circulation, and seemed to be without clinical significance.

# THE ELECTROCARDIOGRAM AND BLOOD PRESSURE DURING SURGICAL OPERATION AND CONVALESCENCE

## EFFECT OF ROUTINE PREOPERATIVE DIGITALIZATION *

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The influence of preoperative digitalization on the incidence of postoperative complications and deaths due to "circulatory failure" is a matter of considerable interest to most surgeons, but one which has apparently received little systematic investigation. A number of observers advocate the routine employment of the drug in certain types of cases or operations, and feel that it is of undoubted value. Thus, Lilienthal,[1] in discussing resection of the lung for suppurative infections, says:

> With or without sepsis, it is the power of the heart to adapt itself which is perhaps the greatest factor in determining resistance. While this is practically so in all surgery, it appears to be more striking in resection of the infected lung. . . In any event, digitalization should be accomplished in the forty-eight hours preceding operation.

Similarly, Thomas,[2] in his discussion of the factors responsible for mortality after prostatectomy, says:

> For years I have digitalized these (prostatectomy) patients as a routine before and after operation, and thereby avoid in many cases drops in pulse pressure that not infrequently prove fatal in undigitalized patients who have been operated on. Not only will this practise save many lives, but it will surely prevent many postoperative complications and promote convalescence.

The presence of arteriosclerosis is regarded as an indication for preoperative digitalization by Richardson,[3] who writes: "In spite of the modern skepticism about the use of digitalis in patients with arteriosclerosis, I feel sure that it is of considerable value in tiding over the period of depression that often follows operations on old people." That the drug may be effective in lessening postoperative pneumonia is the

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* From the Department of Internal Medicine, Yale University School of Medicine, and the New Haven Hospital.

1. Lilienthal, H.: Resection of the Lung for Suppurative Infections, Ann. Surg. **75**:257 (March) 1922.
2. Thomas, B. A.: Factors Responsible for Reduction of Mortality and Morbidity in Prostatectomy, J. A. M. A. **82**:281 (Jan. 26) 1924.
3. Richardson, F. L.: Heart Lesions in Anesthesia, Am. J. Surg. (Anesthesia Supplement) **33**:109 (Oct.) 1919.

belief of Elwyn,[4] who thinks "it is probable that hypostatic congestion of the lungs may at times be prevented by the preoperative use of digitalis, especially in cases with a tendency towards myocardial failure."

Statistical evidence in favor of preoperative digitalization has been presented by several authors. Mandl [5] feels that he secured a reduction of postoperative pulmonary complications from 27 to 8 per cent. by the use of digitoxin before operation. Geist and Somberg [6] have noted postoperative complications in only 2.9 per cent. of their sixty-nine digitalized patients, as against 26 per cent. in thirty-nine similar patients who had not received digitalis. Geist and Goldberger,[7] in a later article, were able to report a further reduction of such complications to 2 per cent., which they attributed to preoperative digitalization, as it is much lower than the average in undigitalized patients.

Examples of similar views as to the efficacy of digitalis in surgical patients could be multiplied without difficulty. Most authors who recommend the frequent or routine practice of preoperative digitalization attribute its beneficial effects to its action on the blood pressure; more specifically, to its action in maintaining the pulse pressure. But with the exception of the papers of Geist and his collaborators, we have been unable to find any studies of the postoperative blood pressure in digitalized patients.

In an attempt to arrive at more exact knowledge of the influence of the drug on patients subjected to anesthesia and operation, we have studied a group of thirty persons who had received full therapeutic doses of digitalis before operation. The method of study has been given in detail in the preceding paper. Briefly, it consisted of determining the blood pressure and taking electrocardiograms at frequent intervals throughout operation, and observing any factors in the administration of the anesthetic or the surgical procedure which might be responsible for changes. After operation, the blood pressure was determined at regular intervals every day for at least ten days, and careful notes made as to the progress of convalescence and the occurrence of complications. The only selection of patients was such as to ensure a wide variety of ages and surgical conditions; as in the previous study, an attempt was made to secure only the more serious operations.

Within the two or three days preceding operation, each patient received by mouth the full amount of the calculated therapeutic dose of

4. Elwyn, H.: Postoperative Pneumonia, Anesthesia & Analgesia **3**:43, 1924.

5. Mandl, F.: Wien. klin. Wchnschr. **34**:214 (April 28) 1921; abstr., J. A. M. A. **77**:79 (July 2) 1921.

6. Geist, S. H., and Somberg, J. S.: Preoperative Digitalization: A Method to Reduce Postoperative Complications, Am. J. Obst. & Gynec. **4**:135 (Aug.) 1922.

7. Geist, S. H., and Goldberger, M. A.: Effect of Preoperative Digitalization in Reducing Postoperative Complications, Ann. Surg. **78**:693 (Dec.) 1923.

digitalis. Dosage was calculated on the basis of body weight, 1.5 gm. per hundred pounds being given. The only form of the drug employed was a carefully standardized powdered leaf which has been in constant use in the medical wards of this hospital. In several instances, operation was postponed after complete digitalization had been effected; in these patients, the digitalis effect was maintained by a daily dose of 0.2 gm. until the morning of operation. None was given afterward except to patients with auricular fibrillation. No other change was made in the usual preoperative preparation, which was the same as for nondigitalized patients.

Evidence of the action of digitalis was secured from the electrocardiograms, which showed the characteristic inversion of the T wave or the S-T interval in twenty-nine of the thirty cases. The one patient whose electrocardiogram did not show the usual effect has been included because he received the proper amount of the drug at an adequate interval before operation, and there was no apparent reason for its improper absorption; it is posible that the T wave in this individual was previously inverted, and had been made upright by digitalis. The change in his tracings during operation, however, has been disregarded in view of the doubt concerning digitalization.

The observations here reported are primarily for comparison with those given in the preceding paper; they have therefore been classified in the same manner. It should be noted that the same rules have been followed throughout: all blood pressures have been classified with reference to their relation to the preoperative level, and differences of 4 mm. or less have been disregarded. The transient rise in pressure which occurred, in most patients, during the induction of general anesthesia has also been disregarded for purposes of classification.

### BLOOD PRESSURE DURING OPERATION

The record of one patient has been omitted, as the blood pressure cuff became loose during operation and subsequent observations were unsatisfactory. Of the twenty-nine patients whose records were complete, the blood pressure during operation was consistently above the preoperative level in thirteen, constantly below in four, fluctuating above and below the preoperative pressure in seven, and at the preoperative level in five. The relation of these changes to the type of anesthesia is shown in Table 1.

TABLE 1.—*Blood Pressures During Operation in Twenty-Nine Cases* *

| Anesthesia | Above | Below | Same | Fluctuating |
|---|---|---|---|---|
| Gas-oxygen (nitrous oxid) | 11 | 2 | 4 | 5 |
| Procain | 1 | 1 | .. | 2 |
| Ether | 1 | 1 | 1 | .. |

* As compared with the preoperative blood pressure.

Five patients were in surgical shock at the conclusion of the operation; in one of them, it followed sudden profuse hemorrhage. It should be pointed out that in four patients the shock was of late development, occurring in several of them at the time of closure, and that all of these had blood pressures above the preoperative level until the rather sudden fall which preceded shock. These four cases have been included in the group with those whose pressures were constantly above the preoperative level. The fifth patient who developed shock had a steady fall in pressure from almost the beginning of the operation.

### BLOOD PRESSURE DURING THE THIRTY HOURS AFTER OPERATION

The records of six patients (Cases 3, 8, 11, 19, 22 and 28) have been omitted from the following analysis; one because of sudden death five hours after operation, the other five because they were in shock at the conclusion of the operation, and their blood pressures were below the preoperative level at practically all observations during the thirty hours following. (Records of patients in shock were also omitted from the corresponding table in the preceding study.) The relation of the systolic and the pulse pressures after operation to the corresponding preoperative pressures is shown in Table 2; it is of interest to observe that the administration of digitalis did not prevent a postoperative fall in systolic pressure in a considerable percentage of the patients. The figures with regard to changes in pulse pressure are even more striking; approximately 40 per cent. of the group showed a fall in pulse pressure after operation. These findings are in sharp contrast to those of Geist and Goldberger,[7] who report that in their digitalized patients "the average drop was found to be only 3 mm. within the first twelve hours, after which the tension gradually returned to normal."

TABLE 2.—*Blood Pressures During the Thirty Hours After Operation* *

| | Systolic Pressure | | | Pulse Pressure | | |
|---|---|---|---|---|---|---|
| | Above | Below | Same | Above | Below | Same |
| 6 hours after operation | 6 | 9 | 9 | 6 | 9 | 9 |
| 18 hours after operation | 11 | 7 | 6 | 7 | 11 | 6 |
| 24 hours after operation | 10 | 7 | 7 | 8 | 9 | 7 |
| 30 hours after operation | 7 | 9 | 8 | 7 | 11 | 6 |

* As compared with the preoperative systolic and pulse pressures.

It is difficult to show a relation between the observed changes in pressure and the clinical course of the patient. Both systolic and pulse pressures varied considerably in individual cases; not infrequently, a patient whose pressure was below the preoperative level at the end of six hours showed a rise to or above that level at the end of eighteen hours, and vice versa. However, the clinical records show that the six patients

whose systolic or pulse pressures were above the preoperative level at the end of six hours had rapid and uneventful recoveries; of the nine patients who showed a fall in pressure at that time, one had protracted nausea and vomiting, one died on the sixth day of pneumonia, and seven had normal convalescences. With regard to the patient who died of pneumonia it should be pointed out that at three of the four observations during the first thirty hours after operation the blood pressure was at or above the preoperative level.

### GENERAL POSTOPERATIVE BLOOD PRESSURES

Three patients died within two days after operation, so only twenty-seven records are available for analysis. From the second postoperative day until the end of the period of observation, the blood pressure was above the preoperative level in four patients, below in twenty, the same in two, and fluctuating within wide limits in one. The interesting feature of this observation, as of the preceding ones, is the large number of patients whose pressure fell and remainded below its normal level.

### COMPLICATIONS

As surgical shock occurred during, rather than after, operation in all cases, it has not been included in the postoperative complications, even though it was apparently the direct cause death in one case (Case 19). One patient died suddenly five hours after being removed from the operating room; she had appeared to be in excellent condition fifteen minutes earlier. The suddenness of death suggests a vascular accident, but necropsy was not permitted. Two patients (Cases 20 and 22) developed postoperative pneumonia on the sixth and the second day, respectively; it proved fatal in both instances. In both, it followed long operations, one performed under ether anesthesia, the other under gas-oxygen. Study of the postoperative blood pressure in these cases discloses no distinctive changes.

A summary of the observation on thirty patients, with changes in blood pressure during and after operation, is given in Table 3.

### CHANGES IN ELECTROCARDIOGRAMS

Eleven patients (37 per cent.) showed, during operation, changes in the electrocardiogram which had not been present in the preliminary tracings. Sino-auricular tachycardia, even though it attained a high grade, has not been included as an abnormality. The observed changes are briefly discussed below:

*Premature Beats.*—Ectopic beats of ventricular origin were noted only once; they occurred in an elderly patient who presented signs of arteriosclerotic heart disease and auricular fibrillation.

TABLE 3.—*Blood Pressures in Digitalized Cases*

| Case | Age | Operation | Length of Operation | | Anesthesia | Preoperative Blood Pressure | | General Operative Blood Pressure | Postoperative Blood Pressure | | | | | | | | General Postoperative Blood Pressure | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 6 Hr. | | 18 Hr. | | 24 Hr. | | 30 Hr. | | | |
| | | | Hr. | Min. | | Systolic | Diastolic | | Systolic | Diastolic | Systolic | Diastolic | Systolic | Diastolic | Systolic | Diastolic | | |
| 1 | 29 | Nephrectomy | 3 | 5 | Gas-oxygen | 96 | 60 | Above | 100 | 62 | 110 | 74 | 112 | 70 | 108 | 70 | Above | |
| 2 | 54 | Nephrectomy | 1 | 20 | Gas-oxygen | 210 | 100 | Below | 184 | 112 | 228 | 146 | 222 | 115 | 130 | 120 | Below | Obesity; hypertension |
| 3 | 68 | Exploration | 1 | 18 | Gas-oxygen | 122 | 78 | Below | ... | ... | ... | ... | ... | ... | ... | ... | ..... | Died 5 hours after operation |
| 4 | 71 | Cystotomy | 1 | 5 | Gas-oxygen | 150 | 70 | Above | 130 | 75 | 150 | 80 | 144 | 72 | 148 | 84 | Below | Arteriosclerotic heart disease with auricular fibrillation |
| 5 | 61 | Cystotomy | 2 | 25 | Gas-oxygen | 150 | 110 | Above | 148 | 92 | 138 | 90 | 134 | 86 | 130 | 86 | Below | |
| 6 | 54 | Hysterectomy | 2 | 15 | Gas-oxygen | 134 | 74 | Same | 130 | 74 | 122 | 74 | 130 | 72 | 128 | 78 | Below | |
| 7 | 37 | Hysterectomy | 1 | 35 | Gas-oxygen | 118 | 68 | Fluctuating | 110 | 74 | 120 | 78 | 122 | 78 | 120 | 80 | Below | |
| 8 | 35 | Hysterectomy | 2 | 25 | Gas-oxygen | 112 | 70 | Above | 88 | 70 | 106 | 88 | 104 | 76 | 88 | 70 | Below | Shock |
| 9 | 27 | Hysterectomy | 1 | 30 | Gas-oxygen | 106 | 70 | Above | 108 | 70 | 114 | 82 | 104 | 86 | 114 | 78 | Above | |
| 10 | 24 | Hysterectomy | 1 | 40 | Gas-oxygen | 130 | 70 | Same | 108 | 48 | 136 | 80 | 134 | 78 | 132 | 74 | Below | |
| 11 | 63 | Cholecystectomy | 2 | 45 | Gas-oxygen | 118 | 86 | Above | ... | ... | 114 | 90 | 112 | 86 | 112 | 80 | Below | Shock following hemorrhage |
| 12 | 48 | Cholecystectomy | 2 | .. | Gas-oxygen | 116 | 70 | Above | 140 | 78 | 140 | 72 | 128 | 70 | 120 | 68 | Same | |
| 13 | 39 | Cholecystectomy | 1 | 30 | Gas-oxygen | 130 | 80 | ..... | 122 | 82 | 118 | 84 | 118 | 82 | 124 | 92 | Below | |
| 14 | 47 | Cholecystectomy | 2 | .. | Gas-oxygen | 120 | 76 | Fluctuating | 130 | 80 | 144 | 92 | 144 | 88 | 142 | 92 | Below | Deeply jaundiced |
| 15 | 51 | Cholecystectomy | 1 | 40 | Ether | 164 | 98 | Below | 162 | 108 | 164 | 112 | 172 | 122 | 162 | 110 | Above | |
| 16 | 15 | Appendectomy | 1 | 5 | Ether | 130 | 76 | Above | 130 | 78 | 128 | 80 | 130 | 76 | 128 | 76 | Below | |
| 17 | 25 | Appendectomy | 1 | 25 | Gas-oxygen | 122 | 80 | Fluctuating | 122 | 68 | 132 | 66 | 132 | 72 | 124 | 74 | Below | |
| 18 | 41 | Herniotomy | 4 | .. | Local | 138 | 98 | Fluctuating | 144 | 100 | 138 | 96 | 138 | 94 | 134 | 98 | Below | |
| 19 | 64 | Resection of stomach | 2 | 45 | Local | 112 | 80 | Steady fall | Systolic blood pressure below 85 | | | | | | | | Died on next day | Arteriosclerotic heart disease; extensive carcinoma stomach |
| 20 | 49 | Resection of stomach | 4 | 10 | Gas-oxygen | 146 | 90 | Fluctuating | 134 | 84 | 142 | 92 | 144 | 90 | 152 | 90 | Below | Died of pneumonia, sixth day |
| 21 | 40 | Resection of stomach | 3 | 5 | Gas-oxygen | 130 | 70 | Above | 126 | 68 | 140 | 80 | 150 | 80 | 134 | 72 | Above | Chronic ulcer |
| 22 | 38 | Brain tumor | 3 | .. | Ether | 168 | 98 | Same | 134 | 82 | 140 | 76 | 170 | 100 | 148 | 82 | ..... | Shock; died on second day |
| 23 | 68 | Amputation of breast | 2 | 55 | Gas-oxygen | 160 | 100 | Fluctuating | 120 | 62 | 128 | 62 | 138 | 70 | 140 | 72 | Below | |
| 24 | 28 | Ventral suspension of uterus | 1 | 25 | Gas-oxygen | 96 | 54 | Above | 126 | 94 | 116 | 82 | 114 | 78 | 110 | 76 | Fluctuating | |
| 25 | 48 | Perineal repair | 1 | 25 | Gas-oxygen | 142 | 94 | Above | 120 | 64 | 132 | 70 | 130 | 62 | 114 | 66 | Below | |
| 26 | 15 | Osteotomy | 2 | 30 | Gas-oxygen | 130 | 68 | Same | 132 | 82 | 134 | 88 | 136 | 80 | 124 | 78 | Below | |
| 27 | 19 | Reduction, testis | 1 | .. | Gas-oxygen | 130 | 68 | Same | 126 | 64 | 114 | 68 | 114 | 70 | 112 | 68 | Below | |
| 28 | 24 | Partial thyroidectomy | 2 | 25 | Gas-oxygen | 150 | 85 | Above | ... | ... | 148 | 82 | 158 | 74 | 148 | 74 | Same | Shock |
| 29 | 63 | Ligation, superior thyroid artery | .. | 55 | Local | 210 | 78 | Fluctuating | 200 | 78 | 202 | 76 | 196 | 70 | 175 | 68 | Below | Hypertension; auricular fibrillation; toxic goiter |
| 30 | 22 | Cardiolysis | 1 | 40 | Local | 115 | 70 | Above | 124 | 80 | 128 | 86 | 126 | 82 | 114 | 80 | Below | |

*Reversal of T Wave.*—In four patients, there occurred a partial or complete reversal of the T Wave in Lead II, which had become inverted through the action of digitalis. The change was transient, but, in every instance, it recurred at intervals throughout operation. Without exception, it appeared in association with a notable increase in heart rate; when the rate fell to its usual level, the T wave became again inverted. All patients who showed this reversal were anesthetized with nitrous oxid and oxygen. Age had no apparent influence on its incidence as the ages of the patients varied from 15 to 48 years. That the change may have been due to vagal release is suggested by its invariable association with an increased rate of beating. The character of the reversal is shown in Figure 1, *A* and *B*.

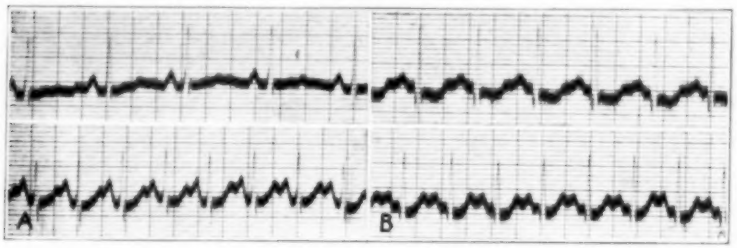


Fig. 1.—Lead II: change in T wave and S-T interval in lower tracings. Increase in rate in *A* is from 90 to 166 a minute; in *B*, from 124 to 162. In this and the following electrocardiograms, distances between abscissas represent $10^{-4}$ volts, and time is in one-fifth seconds.

*Paroxysmal Tachycardia.*—Paroxysms of auricular tachycardia were registered in three patients; one of them also had paroxysms of ventricular origin. From the standpoint of the anesthetic employed, these cases afford an interesting contrast to those mentioned in the preceding paper. The two patients in that series who developed paroxysmal tachycardia both received ether through a nasal tube introduced into the pharynx; of the three patients in the present series, one was anesthetized with nitrous oxid and oxygen, and two with procain. With regard to possible factors concerned in the etiology of the paroxysms, it should be noted that two patients had unequivocal signs of heart disease, one of arteriosclerotic and the other of rheumatic origin, while the third had shown for a long period the continuous tachycardia associated with exophthalmic goiter. Two of the patients developed profound shock during operation, but no paroxysms were noted clinically or in the electrocardiograms after the blood pressure had fallen below 80 mm. of mercury. The tracings of two patients, are reproduced in Figures 2 and 3.

Fig. 2.—Lead II: upper tracing shows normal mechanism, with some depression of the S-T interval; middle record shows sino-auricular tachycardia, with a rate of 184 a minute; lower tracing was taken during a paroxysm of tachycardia, the rate being 202.

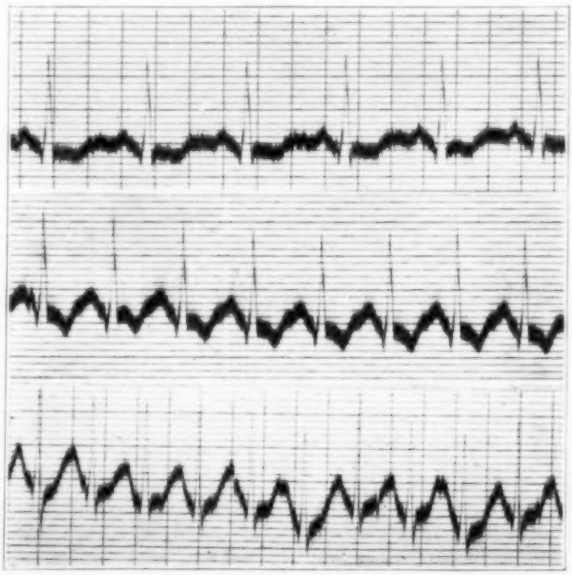

Fig. 3.—Lead II: upper shows normal mechanism, with depression of the S-T interval; middle shows a deep inversion of the T wave during operation; lower is thought to represent a paroxysm of tachycardia, although it is possibly sino-auricular tachycardia, with reversal of the previously inverted T waves, the rate being 180 a minute.

*Displacement of the Pacemaker.*—A disturbance of the pacemaker, as evidenced by a disappearance of the P wave in the electrocardiogram, was noted in three cases. In none of them were there coincident alterations in any other deflections. In no instance was there any apparent change in the circulation or blood pressure which could be attributed to the altered cardiac mechanism. It is true that, in all three cases, the blood pressure was above the preoperative level at the time the abnormal tracings were secured, but the elevation in pressure had occurred before the abnormality appeared in the electrocardiograms, and persisted after normal mechanism had been restored. All three patients were males; all received nitrous oxid and oxygen as the anesthetic. As in previous examples, the change in the electrocardiogram appeared only during the earlier part of the operation.

TABLE 4.—*Electrocardiograms and Blood Pressures*

| | | | Preoperative Blood Pressure | | | Simultaneous Blood Pressure | | |
|---|---|---|---|---|---|---|---|---|
| Case | Age | Cardiac Diagnosis | Systolic | Diastolic | Abnormalities in Electrocardiograms | Systolic | Diastolic | Remarks |
| 4 | 71 | Arteriosclerotic heart disease; auricular fibrillation | 150 | 70 | Ectopic ventricular beats | 180 | 96 | |
| 5 | 61 | Normal | 150 | 110 | Displacement of pacemaker | 188 | 104 | |
| 10 | 24 | Normal | 130 | 70 | Reversal of T wave | 128<br>140 | 72<br>70 | |
| 12 | 48 | Normal | 116 | 70 | Reversal of T wave | 140<br>126 | 78<br>70 | |
| 14 | 47 | Normal | 120 | 76 | Displacement of pacemaker | 148<br>142 | 90<br>98 | |
| 19 | 64 | Arteriosclerotic heart disease | 112 | 80 | Paroxysmal tachycardia (auricular and ventricular) | 96<br>92 | 76<br>74 | Carcinoma of stomach |
| 20 | 49 | Normal | 146 | 90 | Displacement of pacemaker | 156<br>164 | 92<br>98 | |
| 21 | 40 | Normal | 120 | 70 | Reversal of T wave | 138<br>144 | 78<br>76 | |
| 26 | 15 | Normal | 130 | 68 | Reversal of T wave | 128<br>138 | 80<br>86 | |
| 28 | 24 | Thyroid heart disease (?) | 150 | 85 | Paroxysmal tachycardia (auricular) | 122 | 96 | Thyrotoxicosis |
| 30 | 23 | Rheumatic heart disease | 110 | 60 | Paroxysmal tachycardia (auricular) | 154<br>124 | *<br>* | Adherent pericardium |

* Diastolic pressure questionable as sounds were audible without inflation of cuff.

### RELATION OF ELECTROCARDIOGRAPHIC CHANGES TO BLOOD PRESSURE

So far as could be observed, there was no relation whatever between the blood pressure and changes in cardiac mechanism. This complete absence of correlation was found to apply not only to such minor and transient changes as alterations in the T wave and occasional premature beats, but also to such profound disturbances as prolonged paroxysms of tachycardia. In only one instance was there an apparent relation between an abnormal mechanism and a change in blood pressure: the onset of a paroxysm of tachycardia (as determined by auscultation and

graphic records) seemed to be accompanied by a sharp rise in pressure, but similar rises occurred several times after the end of the paroxysm, and a subsequent episode was not associated with an alteration in the blood pressure.

A summary of the electrocardiographic changes and the associated alterations in blood pressure is given in Table 4. Where two blood pressures are recorded, they represent the readings taken simultaneously with the first and last electrocardiograms that showed the abnormality.

### RELATION OF ELECTROCARDIOGRAPHIC CHANGES TO CARDIAC PATHOLOGY

Only four of the thirty patients studied had clear signs of heart disease (Cases 4, 20, 29 and 30), while a fifth (Case 28) was thought to have early changes in the heart of the type associated with some instances of exophthalmic goiter. Of these five, four showed changes in the electrocardiogram during operation. Paroxysmal tachycardia unquestionably stands first in importance among the changes found in this study, and it is worthy of note that of the three patients whose records displayed this abnormality, two had definite heart disease and the third very probably had myocardial damage. There were symptoms and signs of slight congestive heart failure in two cases (Cases 20 and 29); none gave a history of anginal pain.

It is thus seen that four of the five cardiac patients (80 per cent.) developed some abnormality of the heart's mechanism during operation, while only seven of the twenty-five normal patients (28 per cent.) showed any changes, and these were extremely slight.

### SIGNIFICANCE OF ELECTROCARDIOGRAPHIC CHANGES

Five patients developed the picture of surgical shock during operation; two of them had shown paroxysms of tachycardia earlier. The relation of the arrhythmia to the subsequent development of shock seems problematical, for one of these patients had degenerative changes in the heart and a systolic blood pressure below 100 mm. of mercury at the time operation was commenced, while the other had a severe form of exophthalmic goiter; a serious operative and postoperative course was therefore to be expected in both. Furthermore, in one patient, the paroxysms were first detected after the fall in blood pressure was noted, and, in the other, the paroxysms had ended about twenty minutes before the pressure began to decline. It is unlikely that paroxysms occurred which escaped detection, for the condition of the latter patient was so critical that the heart rate was counted through the blood pressure attachment at intervals of about one minute, and electrocardiograms were taken at every indication of a change.

There was no evidence obtained to indicate that the other abnormalities in the electrocardiograms were of the slightest clinical significance.

### COMPARISON OF DIGITALIZED AND UNDIGITALIZED PATIENTS

With respect to the period of anesthesia and operation, blood pressures which fluctuated above and below the preoperative level were noted in a considerable number of the patients who received digitalis, as compared with those who did not. The general level of the blood pressure during operation was above the preoperative pressure in a larger proportion of the digitalized than of the nondigitalized patients. There appears to be a measurable difference between the two groups in these respects, but, in estimating the value of digitalis, it should be remembered that such differences in pressure have seemed to be without clinical significance.

It is with regard to the changes in blood pressure during the first thirty hours after operaion that the most interesting observations have been made. There is apparently a widespread assumption that many postoperative complications and deaths are due to a failure of the circulation which manifests itself objectively through a fall in the systolic pressure or pulse pressure or both. We have already mentioned our failure to find any such postoperative fall in the majority of nondigitalized patients, even in those who developed pulmonary or other complications during convalescence. Of the digitalized patients, however, a high percentage showed a significant lowering of both systolic and pulse pressures during the thirty hours after operation. The actual percentage of the patients in each group who showed this postoperative fall in systolic and pulse pressures is shown in Table 5.

TABLE 5.—*Percentage of Patients Showing Postoperative Fall in Systolic and Pulse Pressures*

| | Systolic Pressure | | Pulse Pressure | |
|---|---|---|---|---|
| | Nondigitalized, per Cent. | Digitalized, per Cent. | Nondigitalized, per Cent. | Digitalized, per Cent. |
| 6 hours after operation | 30 | 37.5 | 34.6 | 37.5 |
| 18 hours after operation | 19 | 29 | 30 | 46 |
| 24 hours after operation | 24 | 29 | 32 | 37.5 |
| 30 hours after operation | 11.5 | 37.5 | 16.6 | 46 |

The changes in blood pressure from the second postoperative day onward have attracted little attention from previous observers, but in this period also there is found the same relative difference in the two groups. Nearly three-fourths (73 per cent.) of the digitalized patients had blood pressures during convalescence well below the preoperative level, while only 43 per cent. of the nondigitalized patients showed a similar lowering.

Concerning changes in the electrocardiogram during operation, the figures appear to indicate that abnormalities occurred less frequently in

the digitalized patients; also that displacements of the pacemaker were less frequent, and paroxysmal tachycardia more frequent, in this group. The differences in the two groups, however, are slight, and no significance can be attached to them when the method of study is considered. Electrocardiograms were taken from most patients only once every five minutes, and the abnormalities rarely appeared in more than two of the twenty or thirty records secured. The actual incidence of changes could be determined only by means of continuous tracings; an apparent slight reduction in the number of abnormalities, therefore, cannot safely be accepted as indicating an actual reduction. That the observed changes were without apparent clinical significance has already been mentioned.

With regard to complications and deaths in the two groups, it is difficult to make a satisfactory comparison. Mere figures indicating the number of postoperative complications and deaths are available, but cannot justify any conclusions. For instance, pneumonia occurred in four nondigitalized patients and in two who had received digitalis; it seems unfair, however, to maintain that the incidence of that disease is twice as great in the first group as in the second, because two of the four patients in the first group who developed pneumonia had prolonged and extensive operations on the jaw, tongue, pharynx and neck, with unquestionable inhalation of septic material. It is hardly conceivable that the preoperative administration of digitalis would have prevented the development of pneumonia in either of these patients. Similarly, we find recorded four deaths among the digitalized patients, as against two in the nondigitalized group, but analysis of the histories reveals that of the four digitalized patients who died, one had advanced heart disease and one a practically inoperable brain tumor. A third patient died suddenly and the cause was undetermined. The behavior of the blood pressure has been of no value in either group. Surgical shock, which has seemed to us an operative, rather than a postoperative, complication, occurred an equal number of times in both groups.

A careful consideration of all the known factors in those patients who developed postoperative complications seems to warrant the conclusion that their incidence in both the digitalized and undigitalized patients was about what would have been anticipated, and that it affords no evidence of a beneficial influence on the part of digitalis.

## COMMENT

It is obviously impossible to draw any final conclusions from a study which has included a comparatively small number of patients and a wide variety of ages and operations. The observations here reported may not be strictly comparable with those of previous authors, but they

are fairly comparable with those given in the preceding paper. The patients who composed the two contrasting groups were drawn from the same wards, were under the same preoperative and postoperative regimen, were anesthetized and operated on by the same group of anesthetists and surgeons. All observations were made by the authors. It is therefore of some interest to find that the preoperative administration of digitalis has not only failed to prevent a postoperative fall in systolic blood pressure and pulse pressure, but has possibly caused such a fall in a large number of patients. It has had no apparent influence on the incidence or severity of postoperative complications. To conclude that digitalis is without value in preventing such complications would be to go beyond the observed facts; it can probably be said with fairness, however, that this study has afforded no support for the belief that there are important changes in the blood pressure after operation which can be prevented by the earlier administration of digitalis. Whether observations on a large group of elderly patients subjected to the same anesthetic and operation would yield similar results is open to question.

SUMMARY

The blood pressure was determined, and electrocardiograms taken, at frequent intervals during anesthesia and operation in thirty patients who had been completely digitalized. Blood pressures were determined and notes of the clinical condition made at regular intervals every day for from eight to eighteen days after operation.

The blood pressure, during operation, varied considerably in individual cases. A large proportion (45 per cent.) maintained the pressure above the preoperative level, and about one-fourth showed wide fluctuations above and below the preoperative pressure.

A considerable number of the patients showed a fall in the systolic blood pressure during the thirty hours following operation. An even larger number showed a decrease in the pulse pressure. These changes seemed to have no relation to the clinical course or the development of complications.

The blood pressure after the second postoperative day was below the preoperative level in more than two thirds of the patients. Its behavior, during this period, was found to be of no value in estimating the rapidity of convalescence.

Electrocardiograms taken during the operation showed changes not present in preliminary records in about 37 per cent. of the patients. The most frequent of these changes were: displacement of the pacemaker, reversal of an inverted T wave in Lead II, and paroxysmal tachycardia. They were unaccompanied by clinical evidences of circulatory disturbance.

A comparison of the observations on digitalized and nondigitalized patients discloses only few differences. There was a postoperative fall in systolic and pulse pressure in a greater proportion of the digitalized patients than of the nondigitalized. No evidence was secured of a beneficial effect of digitalis on postoperative complications or deaths.

## CONCLUSION

There is no convincing evidence that the preoperative administration of digitalis exerts a favorable influence on the blood pressure or the incidence of postoperative complications in a group of unselected patients. There is suggestive evidence that the drug tends to cause the precise change in blood pressure which it is usually given to prevent. There is no apparent necessity or justification for its routine employment in patients who possess normal hearts.

# THE INVOLUNTARY NERVOUS SYSTEM: AN IMPORTANT FACTOR IN THE BODY'S RESISTANCE *

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I.

Infection and body resistance have been studied from various angles for several centuries.

Empirically, nonspecific therapy has been practiced in different forms for an equal period of time, and only of late has scientific endeavor been applied to solve the problems to which the uses of such agents have given rise.

Petersen [1] has particularly emphasized the rôle that body resistance plays in the administration and the results of nonspecific treatment.

Our aim has been to determine the physiologic basis of this resistance; in other words, to point to certain organs whose common functions were practically synonymous with resistance. We believe that resistance is a product of the functions of various organs, and it is quite probable that these organs possess additional functions that are exercised normally in the course of biologic processes. The functions of resistance are normally inherent in the body but, under healthy conditions, their activity is restricted to a minimum; in other words, they may be regarded as latent. When infectious conditions prevail, evidences of their existence become apparent (as in the production of leukocytes and antibodies), but there is nothing to indicate that such an increased function is to be regarded as in any way abnormal or as an abnormal function of the organ involved; on the contrary, it is a natural manifestation of the body's inherent resistance powers.

Our studies were undertaken with the intention of determining (1) the organs of resistance, and (2) how the stimulation to increased activity reaches these organs, and the manner in which this impulse is conducted to the site of infection.

Our experiments to date lead us to the opinion that the entrance of bacterial excitants into the body causes a reaction involving, besides others,[2] chiefly two groups of organs: (1) the bone marrow system,[3]

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1. Petersen, W. F: Protein Therapy and Nonspecific Resistance, New York, Macmillan Company, 1922.

2. Jobling, I. W., and Petersen, W. F.: J. Exper. Med. **22**:141, 1915; Bacteriotherapy in Typhoid Fever, J. A. M. A. **65**:515 (Aug. 7) 1915; Nonspecific Factors, J. A. M. A. **66**:1753 (June 3) 1916. Jobling, J. W.: Nonspecific Substances, Arch. Int. Med. **19**:1042 (June) 1917. Larson, W. P.: Minnesota Med. **2**:332 (Sept.) 1919.

3. Gay, F. P., and Claypole, E. J.: Arch. Int. Med. **14**:662 (Nov.) 1922. Müller, E. F.: Med. Klin. **14**:440, 1918; Beitr. z. Klin. d. Infektionskr. **8**:34, 1919.

the originator of the white blood corpuscles and probably also the source of the carriers of specific resistance (such as agglutinins); (2) the involuntary nervous system,[4] the conductor not only of the stimulation from the site of infection to the bone marrow system but also of the newly formed immunizing substances and leukocytes to the site of infection.

In what follows, reference will be made only to the importance of the involuntary nervous system as a major factor in nonspecific and specific resistance.

In this connection, we accept as a postulate that the vascular system is the principal field of manifested and measurable activity of the involuntary nervous system.

The vasodilatation by the parasympathetic portion of the involuntary nervous system and the vasoconstriction by its sympathetic portion are, under normal conditions, in that state of regulatory equilibrium that is demanded by the nutritive and functional synergism of the organs. We know of influences of various kinds, acting in the main on the body from without, which disturb this equilibrium by giving a preponderating influence to one or to the other of these two antagonists. Less known to us, however, are the *paths* of stimulation on which these impulses of the involuntary system operate and thus lead to changes in the equilibrium of the blood vessel tonus.

Müller and Glaser-Hahnstein [5] point to the everyday occurrence of a unilateral blood vessel innervation by psychic processes and have experimentally shown that the midbrain, the thalamus opticus and the central gray matter of the third ventricle are to be regarded as the places where strong sensory stimuli, as well as those changes in the general irritability caused by changes of mood, pass over to vasomotor channels. They thus discovered one anatomically important part of this path of stimulation, over which pass the vasomotor impulses, whose causes and purpose are still unknown to us.

We may further mention that, pharmacologically, the vasodilatation produced by pilocarpin and the vasoconstriction produced by epinephrin, both of which influences originate in the artificially caused preponderance of one of the factors of the vascular tonus under normal conditions, are at equipoise. For this reason, these alkaloids produce a genuine disturbance in the regulation of the circulatory system. The well known final effect of these disturbances makes it possible for us to ascertain which of the two factors has gained the preponderance in an individual case, and thus points out the right road in the search for the cause.

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4. Müller, E. F.: München. med. Wchnschr. **70**:1163 (Sept. 14) 1923; Arch. f. Dermat. u. Syph. **145**:188, 1923.

5. Müller, L. R.: Autonomes Nervensystem, Berlin, 1923.

Another well known change in the vascular tonus, a vaso dilatation, for the most part strictly localized, is caused by almost all inflammatory irritations. It is true, however, that the question has not as yet been definitely decided whether we have here to deal with genuine disturbances of vasomotor regulation produced by external causes, or whether we must regard this dilation of the blood vessels as a reflex alteration of the local condition of the tissues. The latter explanation is more acceptable, according to the modern view of the problem of inflammation; but the hypothesis of a specific excitation of the vasodilators, put forward by Meyer and Gottlieb,[6] has not been verified as yet with respect to causation or to purpose. On the other hand, it has been experimentally proved, according to the same authors, that a stimulus to the vasodilators is followed, first of all, by an acute hyperemia, and subsequently by an increase in the permeability of the vessels.

II.

Processes in the circulation originating, in the last analysis, from completely different causes, and which may be recognized by signs that have seemingly nothing to do with those previously described, have led us to a new and clearer insight into the regulation of vascular tonus, and have made it possible to explain many well known phenomena.

Goldscheider and Jacob[7] described, as early as 1894, an acute leukopenia resulting from the intravenous injection of peptonoid bodies. These results led to the concept of the "peptone shock" manifesting itself in the blood by a displacement, of short duration, of the leukocytes, principally the neutrophil polymorphonuclears, of which, in this test, a great number disappear for a short time from the peripheral blood and must therefore be retained in the vessels of the inner organs.

In 1920, Widal[8] published an observation, similar in effect. He noted in patients with liver diseases, after alimentary irritation (200 gm. of milk taken on an empty stomach), a leukocyte fall of short duration, accompanied by a slight fall in the blood pressure and alterations in the viscosity and index of refraction of the blood; he designated this phenomenon as "crise haemoclasique." He explains it as follows: Those constituents of food which have neither been assimilated nor completely split up pass normally through the intestinal walls into the blood of the portal vein; they are then retained by the liver, detoxicated,

6. Meyer and Gottlieb: Pharmokologie, Berlin, 1923.
7. Goldscheider and Jacob: Ztschr. f. klin. Med. **25**:373; 582, 1894.
8. Widal, F.; Abrami, P., and Brissaud, D.: Presse méd. **28**:181 (April 3) 1920.

and thus removed from the general circulation. He concluded this was a normally present poison-removing function of the liver and termed it "proteopexic." A disturbance of this function would then cause an acute leukopenia, as a result of the entrance of peptonoid bodies in the general circulation.

This explanation was inconceivable to us because clinical and experimental observations, which will be mentioned later, led to the belief that the leukocyte fall was caused by disturbance of the involuntary nervous system, and we further assumed that this phenomenon is only represented by an acute displacement of the leukocytes.

III.

These conclusions were reached after the following experiments, beginning again from an entirely different angle. In the course of extensive investigations on the rôle of the skin in immunizing processes, which were reported in a series of papers, we observed a new cause for the acute temporary leukocyte impoverishment of the peripheral vessels.[9]

We found that injections of physiologic sodium chlorid solution and other nonirritant substances given intracutaneously led to a dilatation and to an increase in the number of leukocytes in the vessels of the subcutis. On the other hand, after subcutaneous injections, despite the proximity of the vessels, a dilatation never takes place.

These simple experiments prove that it is not the injected material but the place and the manner in which the injection is made into the derma that cause these dilatations of the vessels and the accumulation of leukocytes in the latter.

Thus is proved an influence, originated in the skin, which stimulates the vasodilating nerves of the subcutis. We do not know if such an experiment is equal to an infection produced by germs entering the skin. We further do not know if the reaction of the vessels of the subcutaneous tissues by dilating is a part of a local resistance. In any event, it is the beginning of an active process in connection with the injected agents. Later, it will be observed that leukocytes, accumulating in the dilated vessels of the subcutis, penetrate the walls, enter into the tissues and reach the skin, more particularly the area around the injection.[10]

We further determined, by other experiments, that intradermal injections of minute quantities of physiologic sodium chlorid solution, distilled water, lactalbumin, milk or even air, cause an immediate and

9. Müller, E. F.: Arch. f. Dermat. u. Syph. **31**:237, 1921; München. med. Wchnschr. **68**:912 (July 22) 1921; ibid. **69**:1506; 1753, 1922; ibid. **71**:202 (Feb. 15); 672 (May 23); 813 (June 20) 1924; ibid. **72**:125, 1925.

10. Müller, E. F.: Arch. f. Dermat. u. Syph. **31**:237, 1921.

sudden fall of the leukocytes in all of the vessels of the periphery.[11] These leukocytes will suddenly be found in the strongly dilated vessels controlled by the splanchnic nerve, especially in the liver. These findings have been proved, in recent experiments, by injecting rats with arsphenamin.[12] These observations coincide with the findings of Dean,[13] who discovered a very large number of leukocytes in the dilated liver vessels, more particularly in the smaller, in a patient who suddenly died of an anaphylactic shock. The dilatation of the subcutaneous vessels, situated immediately under the area of injection, as well as the enormous distention of the abdominal vessels, were due to the occurrence of an acute preponderance of the vagotonus, caused by a reflex to the parasympathetic part of the involuntary nervous system.

We succeeded in eliminating this reflex by blocking the parasympathetic nervous system (1) in the skin, and (2) in the abdominal region. It is further possible to bring about the reflex with direct pharmacologic stimulation of the parasympathetic nervous system by pilocarpin or to postpone it by adding epinephrin to the injected fluid. Thus, it was proved that stimulation of the skin changes the balance of the involuntary nervous system not only at the site of injection, but also in the region of the organs controlled by the splanchnic nerve.[14]

More recent experiments demonstrated that the parasympathetic vasodilatation leads to an accumulation of leukocytes at these dilated parts of the circulatory system. Thus has been found the direct relation between the skin and the leukocytes in the blood stream. The existence of this connection and its importance for every skin stimulation is enhanced by the determination that other stimulations, such as freezing, application of heat and Ponndorf's scarification, lead to a like effect on the parasympathetic nervous system.

For example, if in chronic gonorrhea strong or weak stimulations of the skin are employed, an abundance of young and fresh leukocytes are produced and can be found in the excretion of the urethra, where they were previously found only in very small numbers.

The foregoing investigations explain this phenomenon in the following manner: As in every other acute and chronic infection, there is in urethral gonorrhea a local overbalance of the parasympathetic tonus recognizable by the inflammatory vasodilatation. This is in the main similar to local findings subsequent to intradermal injections.

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11. Müller, E. F.: Ztschr. f. d. ges. exper. Med. **32**:120, 1923.
12. Müller, E. F., and Myers, C. N.: Proc. Soc. Exper. Biol. & Med. **21**:474, 1924.
13. Dean: J. Path. & Bacteriol. **25**:3, 1920.
14. Müller, E. F., and Hölscher, R.: Ztschr. f. d. ges. exper. Med. **61**:225, 1924.

While the described skin stimulation is held in balance by the sympathetic nervous system in all vascular regions, with the exception of the site of injection and of the visceral area, this equilibrium is also missing at the site of inflammation, because it is subjected to a constant overbalanced tonus of the local fibers of the parasympathetic nervous system. This overbalance of the parasympathetic tonus is further increased by the sudden stimulation emanating from the skin, and now there occurs the phenomenon that is described above as relating to the increase of leukocytes; the leukocytes in the blood stream are fixed along the walls of the blood vessels, later penetrating the walls and entering the bacterially endangered parts. We are not positive whether this penetration is due to chemotaxis or to impulses inherent in the leukocytes; whichever it may be, the fact remains that they are found in the tissues and in the excretion, where formerly they were absent.

This reaction can also be inhibited by the administraion of epinephrin or atropin and not by pilocarpin, thus proving that an undisturbed parasympathetic system is necessary for this reaction.

It is of further interest that the beginning of the leukocytosis is connected with a similar parasympathetic influence on the dilatation of the blood vessels of the bone marrow system.[15]

No conclusive explanation for the transmission of a stimulative impulse was evident from the findings in the cellular marrow of the vertebrae. More favorable conditions obtain in the fatty bone marrow tissue of the long bones. In acute infectious conditions (for instance, pneumonia), a dilatation of the vessels with resulting hyperemia, followed by vascularization and the first appearance of myeloid elements in what was formerly only fatty tissue, is observed in this area. There is no doubt that this process, an independent reaction of the organism, is based on a change of tonus in the vascular nerves. Vasodilatation, in other words, stimulation by way of the parasympathetic system, predominates, and the assumption is therefore justified that this stimulation, similar to the remote effect on the vessels of the splanchnic area, originates in the vicinity of the site of infection.

From a purely objective point of view, this process is entirely nonspecific, for it is not possible to prove that the ferments contained in the leukocytes and also in the serum can be produced by merely transmitting a kinetic impulse to the myeloid system by way of the involuntary nervous system.

There is only one exception: In acute cases of infectious diseases, as in pneumonia, the cells of the vertebral marrow do not possess any distinct bactericidal properties. The cellular tissues of the long bones,

15. Müller, E. F.: Virchows Arch. f. path. Anat. **246**:50, 1923.

however, which are formed during the progress of the disease, are decidedly bactericidal, thus preventing the excitants, circulating in the blood, from taking a foothold. In this instance, the identical stimulative process furthers the formation of cells with specific properties.

IV.

These tests were not sufficient proof that specific stimulation can also be transmitted along the involuntary nervous system. The following investigations, however, may be accepted as conclusive.

They were made with insulin and rabbits were used for the tests.[16] The tests were carried out as follows: Rabbits were fasted for from eighteen to twenty hours, and their blood sugar was determined. They were then injected with equal quantities of insulin, simultaneously, by both the subcutaneous and the intradermal route.

Banting and Best have designated 1 unit of insulin (per kilogram of body weight) as the quantity required for subcutaneous injection to decrease the blood sugar of a normal rabbit from about 0.1 to 0.11 per cent. to about 0.045 per cent. This has since been accepted as the physiologic unit for insulin treatment.

In comparing the results of the subcutaneous and the intradermal methods of administration, it will be found that the effect of an equal dose of insulin is considerably prolonged if given by the intradermal route. This is rather remarkable and difficult to explain. It should be noted that factors of resorption alone could not explain these variations, for practically the same effect on the blood sugar is evident with both methods within a period of two hours. If, after intradermal injection only part of the injected insulin can take effect immediately, due to slow resorption, surely the effect on the blood sugar cannot be equal to that produced by the larger quantity of insulin that is absorbed immediately after subcutaneous injection.

Assuming that a subcutaneous dose is resorbed within thirty minutes after administration, this would by no means afford an explanation for the increased effect observed after intradermal injection that is of equal intensity, yet is of two hours' longer duration. In animals after intradermal injection, the wheal disappears comparatively soon; it is never visible longer than thirty minutes. Even if it is assumed that the difference in reaction of the two methods of injection is due to the slower resorption by the intradermal route, it would be expected that the period of initial low sugar would merely be postponed. Furthermore, the delay might be expected to last a little longer than the time during which the wheal is discernible. This explanation becomes

16. Müller, E. F., and Corbitt, H. B.: J. Lab. & Clin. Med. **9**:608 (June) 1924.

improbable when it is considered that the lowest value of the blood sugar for both methods is reached after from one to two hours, the difference existing solely in the period during which this low value is maintained.

Summarizing our experiments: There is evident, after intradermal administration, a considerable prolongation of the effect of insulin on the blood sugar of normal rabbits. At first we were not able to give an explanation of these facts.

Beyond doubt, the skin as an individual organ is partly responsible for this effect; also, the factor of resorption plays an important part. However, the foregoing alone does not suffice to explain this phenomenon.

The following is of importance:[17] The effect of insulin injections increases proportionately to the distance from the circulatory system at which the injection is made; in other words, intravenous injections are the least effective, intramuscular and subcutaneous injections are considerably more effective and intradermal injections have the greatest effect of all.

These findings suggest a comparison with the former tests with nonspecific agents. Their effect also is greatly enhanced by the intradermal method of injection while equal doses administered subdermally are ineffective, and it has been definitely determined that this increased effectiveness is due to the close relation of the skin to the involuntary nervous system.

We were, therefore, led to assume that there exists a similar path of conduction by way of the involuntary nervous system. When we recall the highly interesting investigations of Meltzer and Kleine,[18] this assumption gains in strength. The authors determined that the effect of epinephrin on hyperglycemia is inversely proportional to the rate of its introduction into the circulation; in other words, the effect is only slight after intravenous injection and is considerable after subcutaneous injection. Our most recent, as yet unpublished, investigations indicate that intradermal injection in these cases, in certain limits, will increase or prolong the effect.

Additionally there is the significant determination that even entirely nonspecific agents, such as sodium chlorid solutions, will effectually increase the blood sugar if injected intradermally.

It is quite obvious that former methods of investigation suggested themselves. We therefore attempted to block the involuntary nervous

17. Corbitt, H. B.: J. Am. Pharm. A. **14**:108 (Feb.) 1925. Müller, E. F., and Corbitt, H. B.: J. Lab. & Clin. Med., to be published.

18. Meltzer and Kleine: Rockefeller Inst. Pub. **19**:146, 1914.

system. The results of such tests with epinephrin and insulin are known. Epinephrin diminishes the effect of insulin. There is no doubt that epinephrin increases the tonus of the sympathetic nervous system so that we suspect there may be a connection between these facts. This gains more importance when one remembers that atropin also diminishes the effect of insulin. Magenta and Biazotti [19] arrive at similar results. This may be explained as follows: Epinephrin increases the tonus of the sympathetic nervous system and has a depressing and paralyzing effect on the parasympathetic nervous system. Atropin has, to a certain degree, a directly paralyzing effect on the parasympathetic nervous system. This paralyzing effect either diminishes or nullifies the effect of insulin. It is, therefore, feasible to conclude that the effect of insulin is conducted in part at least along the route of the parasympathetic nervous system.

Pilocarpin has no effect on insulin medication, nor has physostigmin (eserin) or nicotin, according to Magenta and Biazotti. These facts also indicate that the parasympathetic nervous system plays a part in the realization of the insulin effect, for, if the function of the parasympathetic nerves is disturbed, the effect of insulin is greatly diminished, but no stimulation of this system can affect it.

It is not our intention to draw any conclusions regarding insulin treatment and diabetes from the findings of these experiments. However, it is certain that both insulin and epinephrin affect the blood sugar content through a stimulative impulse traveling by way of the involutary nervous system.

At the time of these studies it was not certain whether or not insulin takes effect only in the manner described, but our investigations, as well as those of other authors, have led to the conclusions that the involuntary nervous system conducts a specific stimulation without biologic contact directly to the liver or to some other organ that is responsible for the blood sugar lowering effects.

Later studies on the mechanism of insulin action carried on by the author and Dr. H. J. Wiener in the Department of Metabolism, Vanderbilt Clinic, College of Physicians and Surgeons, New York, will be published shortly in this journal. They demonstrate that in human beings insulin, injected intradermally, acts in part by a nervous stimulation of the glycogenic function of the liver in the absence of demonstrable evidence of the presence of the hormone in the blood stream or in the tissues. These results show that a specific stimulus can be conveyed by the involuntary nervous system from the site of injection to a distant center.

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19. Magenta, M. A., and Biazotti, A.: Compt. rend. Soc. de biol. **89**:1120, 1923.

V.

We have stressed the importance of the involuntary nervous system in connection with the experiments quoted, and we wish to reiterate that the involuntary nervous system is the conductor of certain impulses, that no substance, nothing material, has to pass from one organ as the first recipient of the stimulation, to the other organ, which later accepts and reacts to this stimulation.

Nothing material, merely stimulative impulses, are transmitted. This knowledge of the principles and mechanism underlying the body's resistance, in those instances, offers a new series of problems and also justifies the foregoing point of view on the problem of infection and resistance.

Summarizing, when excitants enter the body, two great systems of organs participate in setting up the ensuing reaction, either alone or in combination with others: One, the bone marrow system, the originator of the properties and the cells prerequisite for resistance, and probably also the originator of the ferments of specific resistance; the other, the involuntary nervous system, the conductor of impulses of stimulation from an infected area to the myeloid system and at the same time the regulator not only of the vessels but also of their leukocytic content.

At the site of infection, we must assume an involuntary perception of the presence of infectious material resulting in impulses that, in turn, produce a dilatation of vessels in the surrounding tissues by means of an increased tonus of the local fibers of the parasympathetic system. Simultaneously with this dilatation there is a change in the normal condition of the walls of the vessels which, as a reflex, results in an increase of leukocytes in these dilated parts.

This early local increase of leukocytes occurs without any involvement of the bone marrow system, and is the result of changes in the walls of the vessels caused by the parasympathetic system enabling the vessels to retain more leukocytes from the circulating blood.

Beside this first increase of leukocytes at the site of infection there is later observed a decided increase in the number of leukocytes in the entire blood stream due to an increased activity of the myeloid system. This produces not only the elementary cells but also their specific ferments.

The involuntary system also is vitally concerned in the production of this typical "myeloid reaction." So far, experimental investigations have not revealed the direct path along which the stimulation travels from the site of infection to the bone marrow. We know the path as far as the vessels of the surrounding tissues are concerned, and we know that from there the parasympathetic system conducts the stimulation to the abdominal vessels. But, from purely anatomic experiences,

there is a high degree of probability that the stimulation is carried from the site of infection to the myeloid system by way of the involuntary nervous system. No objective findings in the bone marrow of the vertebra sufficient to serve as an explanation for the stimulative conduction have been determined. In the fatty tissues of the marrow of the long bones, conditions are more favorable. At this point, there is first observed great dilatation of the vessels filled with blood; this stage is followed by vascularization and, later, by the first appearance of myeloid elements. This dilatation is beyond doubt an independent reaction of the body produced by the nerves regulating vascular tonus. In other words, vasodilating impulses traveling by way of the parasympathetic system predominate, and we are therefore quite justified in assuming that these impulses are sent out from the site of infection, which creates a condition analogous to that of telekinetic influence on the vessels controlled by the splanchnic nerve.

The resulting increase in cell production of the myeloid system may spread to involve all portions of the bone marrow capable of transformation. If this process continues undisturbed, it will produce a total increase of normal, fully developed myeloid cells, presenting the clinical symptoms of typical leukocytosis.

As soon as this normal hyperactivity of the myeloid system increases the number of polymorphonuclear leukocytes in the circulating blood, the involuntary nervous system acts by conducting these newly formed cells to the site of infection. On reaching the dilated vessels—dilated because of the continued increase of tonus in the parasympathetic system of this area—the leukocytes are retained. Thus, the total number of leukocytes in these vessels increases at the same time that they commence to emigrate through the walls of the vessels into the tissues endangered by germs; together with the local tissue reaction, and yet distinctly individual, there are produced the clinical symptoms of an acute local inflammation. This closes the circle: with the aid of the bone marrow and the involuntary nervous system, the body is thus enabled to afford a certain protection to the endangered area.

# DISEASES OF THE PANCREAS

## A PATHOLOGIC STUDY, WITH REPORT OF CASES

MOSES BARRON, M.D.
MINNEAPOLIS

Few diseases associated with definite organic changes present so many pitfalls in diagnosis as certain lesions involving the pancreas, which are either left undiagnosed or are diagnosed incorrectly. These are diseases associated with the function of the external secretion of the gland. There is as much confusion today on the pathology and symptomatology of these diseases as there was twenty years ago.[1] Disorders of the internal secretion which result in diabetes through involvement of the islands of Langerhans are more readily diagnosed.

Several reasons may explain the difficulties encountered in diagnosis: the location of the organ in the center of the body, which makes it inaccessible to palpation and surgery, and also inaccessible to securing the secretion through which to study the function of the organ; its structure and location, which make it inaccessible to the roentgen ray, and the fact that it is not often studied at necropsy, probably because of the early postmortem autolysis.

### PATHOLOGY

There has been a gross exaggeration concerning the infrequency of pancreatic disease, owing to this difficulty in diagnosis, and also to the fact that the disease is not looked for because of the assumption of its rarity.

Mayo-Robson and Cammidge[2] furnish statistics that show more than 2 per cent. of lesions of the pancreas in a series of 6,708 necropsies. They state that postmortem figures in relation to the pancreas are not reliable, because of the rapid autolytic changes taking place soon after death with the result that many lesions remain unrecognized.

The records of all necropsies performed in the department of pathology at the University of Minnesota Medical School, from Jan. 1, 1919, to Sept. 10, 1924, were examined, and seventy-three cases of gross lesions in the pancreas were found. The total number of necropsies for this period was 3,437, of which about 200 were of stillbirths and new-born infants. The percentage of pancreatic

1. Fallon, M. F.: Relation of Gallstones to Pancreatitis, Boston M. & S. J. **190**:545-549 (March 27) 1924.

2. Mayo-Robson and Cammidge: The Pancreas: Its Surgery and Pathology, Philadelphia, W. B. Saunders Company, 1907, p. 126.

disease, in this series, is therefore 2.3 per cent., a result quite similar to that quoted by Mayo-Robson and Cammidge. The card index was used to find pancreatic diseases in the postmortems prior to 1919. Twenty-two additional cases were compiled in this group, making a total of ninety-five cases studied.

The classification of lesions quoted by Mayo-Robson and Cammidge is as follows: Total number of diseases of pancreas found, 142; tumors (primary and secondary), 55; atrophy or cirrhosis, 45; fatty changes, 9; hemorrhage, 3; calculi, 3; cysts, 2; hydatiform cysts, 1; tuberculosis, 4; abscesses, 3, and miscellaneous, 17.

The types of lesions found, in our series, are as follows: Primary carcinoma, 29; melanoma, 1; metastatic carcinoma, 26; chronic pancreatitis, 14; acute pancreatic necrosis, 8; pancreatic lithiasis, 3; traumatic rupture, 4; gangrenous pancreatitis, 2; cyst, 1; hemachromatosis, 1; apoplexy, 1; pancreatic arteriosclerosis with coronary sclerosis, 3; pancreatic arteriosclerosis with sclerosis of kidney and hypertension, 1, and infarction, 1.

Of the primary carcinomas, twenty-two were in men and seven in women. The ages were distributed as follows: Between 38 and 39, three; 40 and 49, three; 50 and 59, twelve; 60 and 69, seven, and 70 and 79, four.

Eighty per cent. were therefore above the age of 50.

Metastases were present in twenty-four cases, distributed as follows; Liver, 20; lymph nodes, 10; lungs, 6; kidney, 4; suprarenal, 3; stomach, 2; gallbladder, 2; heart, 1, and miscellaneous, 5.

Jaundice was present in eleven cases, gallstones in four; the liver was markedly enlarged in nine; the gallbladder enlarged, without stones, in three.

In the twenty-five cases of secondary carcinomas of the pancreas found, the primary locations of the tumor were as follows: Stomach, 19; rectum, 2; duodenum, 2; liver, 1; ovaries, 1, and mediastinal sarcoma, 1.

Of those originating in the stomach, sixteen were in men and three in women. Of this group, gallstones were present in only one case. The age distribution was as follows: Between 30 and 39, two; 40 and 49, three; 50 and 59, four; 60 and 69, seven, and 70 and 79, three.

Seventy-four per cent. of the cases were therefore above the age of 50.

As will be seen from the foregoing, the pancreas is frequently the site of metastatic tumors, especially from the stomach. Of twenty-six cases, nineteen were secondary to gastric carcinoma. Of these, there was a remarkable preponderance of men over women, a ratio of more than five to one. The age incidence of the gastric carcinoma is very similar to that of the pancreatic carcinoma, that is, 74 per cent.

occur after the age of 50. One case of metastatic tumor occurred in a young man, aged 22, in whom the primary carcinoma was in the liver.

Gallstones were present in five cases of chronic pancreatitis, in four cases of acute pancreatic necrosis, in one case of pancreatic stone, and in one case of cyst of the pancreas. These figures present a much higher percentage of gallstones associated with inflammatory lesions of the pancreas than with carcinomas. The percentage of gallstones with primary carcinoma is found to be higher than in secondary carcinoma. Such relationship between lesions of the gallbladder and the primary lesions of the pancreas seems to be more than accidental. Jaundice was present in four cases of chronic, and in one case of acute pancreatitis. Masses were palpable in the epigastrium in one case of chronic and in two cases of acute pancreatitis.

*Tumors.*—Primary neoplasms constitute the most important single group of pathologic conditions involving the pancreas. Benign tumors are rare. Schneider[3] reports two cases of adenoma in addition to mentioning thirteen cases in the literature. In our series, not a single case of benign tumor was found. Of thirty-one cases of primary tumors, twenty-nine were carcinomas, one melanoma and one lymphosarcoma. Pancreatic tumors other than carcinomas are therefore negligible.

Involvement of the head of the pancreas is most frequent. In a large percentage of cases, this condition is accompanied either in the earlier or in the later stages by the development of jaundice, which is often of an intensity that is almost pathognomonic. The gallbladder not infrequently is greatly distended in this type of obstruction in contrast to its shrunken size in cases of gallstone jaundice. It may be large and palpable even in the absence of jaundice. The principal symptoms are indigestion, weakness, progressive loss of weight, anorexia, nausea, vomiting and pain. In our series, about 50 per cent. had gastric distress with nausea and vomiting, and in about 20 per cent. tumor masses could be palpated in the epigastrium. Pain is very common. It is often referred to the lower dorsal or lumbar region and to the angle of the left scapula. Itching is often intense when jaundice is present, as it was in a case which will be referred to later. Gilbride,[4] however, found an absence of itching, in his series of cases of malignant disease. Although the jaundice is usually progressive, occasionally it may clear up after a time, only to recur. In one of the cases studied, the jaundice had completely cleared up for a period of two months.

3. Schneider, Helene: Adenoma of Islands of Langerhans (Insulinoma), Rev. méd. de la Suisse Rom. **44**:222-238 (April) 1924.

4. Gilbride, J. J.: Tumors of the Pancreas: Operations Performed on Twelve Patients, J. A. M. A. **83**:984-989 (Sept. 27) 1924.

The liver very frequently becomes enlarged during some stage of the disease, while it may later shrink to even less than normal in size.

In our series, the ages range between 38 and 75; 80 per cent. occur in those over 50, the largest number occurring between 50 and 60. There is a great preponderance of men over women, which, in our series, is in the ratio of more than three to one.

The duration of the disease ranges from two weeks to four years. The presence of glycosuria was not mentioned in the protocols, except in a few instances. The clinical diagnosis was given in only nine cases, and of these three were diagnosed as carcinoma of the stomach, two as carcinoma of the colon, three as carcinoma of the liver, and only two as carcinoma of the pancreas.

*Acute Pancreatic Necrosis.*—This is a severe acute disease of the pancreas, often called acute hemorrhagic pancreatitis. The hemorrhagic, gangrenous and suppurative forms merely represent different stages in the same pathologic process, which begins with necrosis of the pancreas. The mortality of the disease is very high, variously estimated at 70 per cent., and only a very early surgical intervention may bring the mortality to a lower figure. In our series, there were eight cases of this lesion.

The symptoms of acute pancreatic necrosis are such as generally lead to erroneous diagnoses, such as perforating peptic ulcer, gallbladder disease, acute intestinal obstruction, kidney stone and angina pectoris. Usually, an operation or a postmortem examination establishes the correct diagnosis. The patients attacked are generally of middle age, in our series ranging principally between 30 and 50.

The urine should be repeatedly examined for sugar in such attacks. Rodriguez [5] reports a striking case of severe symptoms in a woman presenting no sugar in the urine, but in whom, three days later, there was a marked glycosuria with 0.6 per cent. blood sugar and abundant acetone and diacetic acid. The patient died in diabetic coma. At necropsy, acute hemorrhagic pancreatic necrosis with suppuration was found.

*Chronic Pancreatitis.*—In our series, there were almost twice as many cases of chronic pancreatitis (fourteen cases) as of acute. There are two forms of chronic pancreatitis, the interlobular, characterized by overgrowth of connective tissue between the lobules, and the interacinous, in which fine bands of connective tissue are scattered between the acini, with more or less atrophy of the gland. In many of these there is present a definite sclerosis of the arteries. When the

5. Rodriguez, Juan: Acute Pancreatitis with Fat Necrosis Complicated by Diabetic Coma, J. A. M. A. **82**:203-204 (Jan. 19) 1924.

atrophy and degeneration involve not only the acini of external secretion but also the elements of the internal secretion, the islands of Langerhans, diabetes results. Intermittent attacks of glycosuria are rather frequent in these cases, and the importance of this finding cannot be overemphasized.

"Chronic pancreatitis" is found much more frequently by surgeons at operation than by pathologists at necropsies. The enlarged, firm head of the pancreas so often found by surgeons at operation and diagnosed as chronic pancreatitis is probably only edema, congestion and exudate of temporary duration. The relative "infrequency," on the other hand, as reported by pathologists at necropsies, may be explained by the fact that the pancreas is rarely carefully studied by them, the organ being dismissed after a casual gross inspection with the report "pancreas apparently normal." Sections of the gland are seldom made. The early autolysis of the pancreas, often within a few hours postmortem, undoubtedly contributes to the carelessness of pancreatic postmortem pathology. It is not unlikely that many and various affections of the gland thus remain unrecognized.

*Pancreatic Calculi.*—Stones of the pancreatic duct are very rare; in our series of more than 3,000 necropsies, only three such cases were encountered. One of these has already been reported in detail in a previous communication.[6] Kretz [7] found only one case in 3,000 necropsies, and Simmonds,[8] one in 2,000. The symptoms of pancreatic stone often closely resemble gallstone colic, but the presence of glycosuria during or immediately following some of the attacks is very helpful for a differential diagnosis. Lanceraux [9] states that temporary glycosuria accompanies each attack of colic. This is undoubtedly an exaggeration. Still, Kinnicutt [10] strongly emphasizes the importance of glycosuria accompanying the attacks of pain as a diagnostic sign differentiating attacks of pancreatic stones from those of gallstone.

The finding of sugar in the urine at various intervals was greatly relied on in the diagnosis of a case reported below. The symptoms at times may be vague, with pain in the epigastrium of different degrees of severity, sometimes agonizing in character, associated with vomiting. The attacks bear no relation to the kind of food or the time of meals.

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6. Barron, M.: The Relation of the Islets of Langerhans to Diabetes, with Special Reference to Cases of Pancreatic Lithiasis, Surg., Gynec. & Obst. **31**:437-448 (Nov.) 1920.

7. Kretz, R.: Handb. d. allg. Path. **2**, 1913, cited by Pratt: Diseases of the Pancreas, Oxford Medicine, Oxford University Press, **3**:473, 1921.

8. Simmonds, M.: Pancreatic Lithiasis, Fortschr. a. d. Geb. d. Röntgenstrahlen **30**:81 (Jan. 15) 1923.

9. Lanceraux, E.: J. de méd. int., February, 1889.

10. Kinnicutt, F. T.: Pancreatic Lithiasis, with Report of a Case, Am. J. M. Sc. **124**:948-956 (Dec.) 1902.

Lazarus [11] reports approximately 50 per cent. of cases of diabetes associated with pancreatic stone in the eighty cases that he compiled from the literature. This fact again emphasizes the importance of glycosuria.

*Cysts.*—Cysts of the pancreas are about as rare as pancreatic stone, only one cyst being found in our series. Pathologically, they may be classified as (1) neoplastic cysts (cyst adenomas), (2) retention cysts and (3) pseudocysts. The last group is the most common. The pseudocyst generally arises through the collection of blood or the production of secretion and digestion in the lesser peritoneal cavity. An attack suggestive of acute pancreatic necrosis or a history of trauma frequently precedes the cyst formation. Pain is the chief symptom. Glycosuria is uncommon. When there is a fluctuating tumor in the epigastrium a little to the left of the midline, with a history as before stated, a diagnosis can readily be made. These cysts generally develop rapidly, in contrast to true cysts, which develop slowly.

*Hemachromatosis.*—Hemachromatosis, or bronze diabetes, is a very rare condition due to a disorder of metabolism of carbohydrate and iron pigment associated with a sclerosis of the pancreas and other glands in the body, together with a deposition of pigment in various tissues and organs. The diagnosis can readily be made when the triad of symptoms, pigmentation of the skin, hepatic enlargement and glycosuria, is present. The diabetes associated with the pigment cirrhosis of the pancreas is probably neither the cause nor the result of the pigment anomaly. The causative factor of the disturbed pigment metabolism is probably also the cause of the diabetes. In our series, there was only one instance of this disease.

*Apoplexy of the Pancreas.*—Apoplexy of the pancreas is extremely rare, so rare that some authors deny its existence. The lesion, in this condition, consists of a massive hemorrhage into the pancreas without any evidence of pancreatic necrosis or other pathology except the sclerosis of the pancreatic blood vessels. The case, in our series, was that of a man, aged 47, who had had advanced hypertension associated with severe headaches and poor vision due to retinal hemorrhages extending over a period of four years. There was albumin in the urine and reduced renal function. About two weeks before death, there was a sudden attack of severe abdominal pain with backache, nausea and vomiting. The blood sugar ranged between 0.12 and 0.18 per cent. At necropsy, there was the usual hypertension heart and a very extensive hemorrhage into and around the pancreas. The hemorrhage extended retroperitoneally to such an extent that intestinal obstruction

11. Lazarus, P.: Beitrage zur Pathologie und Therapie der Pankreaserkrankungen mit besonderer Berucksichtigung der Cysten und Steine, Ztschr. f. klin. Med. **51**:95, 203; **52**:381, 521, 1921.

was produced in two places through pressure. The blood clot in the pancreas itself showed various ages in its formation, suggesting slow bleeding. The pancreatic blood vessels showed atherosclerosis and rupture. This condition is entirely analogous to cerebral hemorrhage.

*Pancreatic Arteriosclerosis.*—A condition which I believe of great clinical importance and which is very little mentioned in textbooks or in the literature, but which may be noted in careful clinical studies, is the rather frequent association of glycosuria and true diabetes in cases of angina pectoris and other types of coronary sclerosis. In our postmortem series, careful studies of the pancreas were noted in three cases of coronary sclerosis, and in these advanced atherosclerosis of the pancreatic arteries was found. In another case of advanced hypertension with arteriosclerotic kidneys, similar changes were found.

When one remembers that one of the types of pathologic changes in the pancreas associated with diabetes is the atrophy of the parenchyma including the islands of Langerhans, as a result of atherosclerotic changes in the blood vessels, it is not surprising that the foregoing relation should obtain. From clinical observations, one is led to conclude that this association of lesions could be demonstrated at necropsies more frequently if more careful histologic studies of the pancreas were made.

The following cases are cited in detail to illustrate the difficulties encountered in arriving at correct diagnoses in cases of carcinoma of the pancreas, pancreatic lithiasis and chronic pancreatitis. Two of these cases, besides having been studied by me, were also studied in one of the leading clinics in the country as well as by a number of physicians.

## REPORT OF CASES

CASE 1.—A. W[illegible] man, aged 45, married, a merchant, when first seen by me, April 8, 1922, [illegible]mplained of pains in the back radiating forward into the abdomen of about [illegible]e or six weeks' duration, constipation, gradual loss in weight and progre[illegible]ive weakness. About five months before, he had been refused insurance because of diabetes, for which he was treated by a diabetes specialist for several months.

The previous history and the family history were entirely negative. The usual weight was 205 pounds (93 kg.); the present weight, 186 pounds (84.4 kg.). Constipation had developed six weeks before. He had been awakened at night by severe pain in the upper part of the abdomen radiating to the back. The principal complaint at the time of examination was backache, at times dull, at other times sharp, which radiated into the epigastrium. There was no nausea or vomiting. Recently, he had had sharp, shooting pains into the left knee and a dull pain with fulness in the abdomen.

Physical examination was entirely negative except for a facial expression of severe distress. Deep percussion over the lower dorsal vertebrae produced pain deep in the epigastrium.

Frequent examinations of the urine showed a small amount of sugar present on two different occasions. While under treatment for diabetes, sugar had been found only five or six times. Examination of the feces and stomach

contents was negative. Because of the persistence of severe pain in the back, a spinal puncture was done. The spinal fluid was negative to all tests. Roentgen-ray studies showed no gastric or duodenal lesions.

Blood sugar estimations gave peculiar results. One examination showed 0.13 per cent. An examination following a rather severe attack of pain gave 0.19 per cent., with a trace of sugar in the urine. A test one month later gave 0.13 per cent.

Because of the history of glycosuria and the essentially negative findings on physical examination, together with the rather erratic sugar findings, both in the urine and the blood, which had no definite relation to the food intake, the probable diagnosis of pancreatic disease suggested itself. A corroboration could not be found in the examination of the stools for excess of fat or undigested muscle fibers. A sugar tolerance curve gave essentially normal results.

In the course of the observation, the patient was seen by a large number of prominent physicians without a definite diagnosis being arrived at. Because of no improvement in his condition, he visited one of the large clinics on two different occasions, when very careful studies were made. They found no definite organic lesions, and concluded that the symptoms were largely due to the neurotic condition of the patient.

The following are a few extracts from my clinical notes on this case:

April 11, there was a severe, gnawing pain in the back, worse on sitting.

May 1, the weight was 182 pounds (82.6 kg.); the condition was worse.

May 29, the pain was still more intense; the weight was 168 pounds (76.2 kg.).

June 28, the pain was more cramplike in character; the weight was 172 pounds (78 kg.).

July 11, the weight was 176 pounds (79.8 kg.). The patient had had agonizing attacks of pain; the urine showed sugar present.

July 11, after two weeks at some mud baths, he returned without relief. The weight was 166 pounds (75.3 kg.). There was an icteric tinge to the conjunctiva and skin, tenderness in the epigastrium, and considerable bile in the urine. A diagnosis of carcinoma of the pancreas was now definitely made and an exploration advised. Operation showed the head of the pancreas large, nodular and cartilaginous, corroborating the diagnosis of carcinoma of the head of the pancreas. The gallbladder was markedly distended; there were no gallstones.

The patient failed rapidly; the skin became intensely greenish-brown, and itching was very marked. He became very emaciated; the liver enlarged to below the umbilicus, then receded again. The patient died, Jan. 18, 1923.

In this case, the early disturbance of the pancreas by carcinoma was manifested by glycosuria. This is less common in carcinomas than in other affections, but nevertheless does occur. Though Joslyn well points out that every case of glycosuria should be considered as diabetes until proved otherwise, every case of glycosuria should not be *treated* as diabetes unless diabetes can be established by further appropriate tests and observations. The irregular and variable sugar levels in the blood on a constant diet and the inconstant presence of sugar in the urine, together with the normal sugar tolerance curve, indicated a disturbance of the pancreas other than true diabetes.

Case 2.—B. B., a man, aged 55, married, when first seen, Dec. 26, 1923, complained of marked weakness, loss of 20 pounds (9 kg.) in weight, attacks of pain and fulness in the epigastrium. The illness was of three months' duration. The family and the past histories were negative. About one year before, he had developed backaches, which lasted for a day or two.

About the middle of September, he developed severe pains, stabbing in character, in the lower part of the right side of the chest, radiating to the right hypochondrium. He was examined in a clinic and the condition diagnosed as probable gallstones. In the latter part of November, he was examined in one of the leading clinics in the country; complete physical and laboratory

examinations led to the diagnosis of probable pleurisy and questionable gallbladder, with no indications for an operation. A roentgen-ray examination of the chest showed a thickened pleura at the right base.

After returning home, he developed a number of very severe attacks of cramps in the upper part of the abdomen, many of them sharp and stabbing in character, lasting from one to three days. He was very ill during the attacks, but between attacks he felt well, except for weakness. About the middle of December, he had a severe attack of pain in the left side of the upper part of the chest anteriorly, lasting one day. During some of the attacks, he would perspire so freely as to drench the bedclothes.

Physical examination showed: Temperature, 99.4 F.; pulse, 94, and respiration, 28. There was definite evidence of loss in weight and marked tenderness in the right epigastric region, but nothing palpable. Deep percussion over the lower dorsal spine produced pain in the epigastrium.

The urine showed three plus sugar. On the following day, sugar was absent. Blood examination showed 37 per cent. hemoglobin and 11,600 leukocytes. The stomach contents and the stools were entirely normal.

December 28, the leukocyte count was 4,900 and blood sugar 0.123 per cent.

December 29, the urine showed no sugar in the morning specimen, 2 per cent. in the evening specimen.

January 17, the patient was improved; the weight was 164 pounds (74.4 kg.).

February 22, a large, tender mass was palpable in the right hypochondrium, conforming to the liver outline.

March 7, the liver was not palpable.

March 26, the patient had a severe attack of backache, cramps, vomiting and chills. There was a mass in the gallbladder region the size of a hen's egg, and an icteric tinge of the conjunctiva. The urine showed 1.6 per cent. sugar, urobilin, urobilinogen and a trace of bile. The fasting blood sugar, two days later, was 0.242 per cent.

April 19, the patient had another severe attack of cramps in the lower part of the abdomen.

May 16, there was definite jaundice of the skin with itching; the liver was large, reaching 8 cm. below the costal margin.

May 22, an exploratory operation showed a soft, mushy mass in the head of the pancreas, not at all suggestive of carcinoma. A cholecystostomy was performed for relief of jaundice. The patient died, four days later, from bronchopneumonia.

This case presents findings of unusual interest. During the first three days of study, the symptoms of colicky pain, deep in the epigastrium and back, together with erratic sugar curves in the urine, suggested very strongly the diagnosis of a lesion in the head of the pancreas, probably stone with acute suppurative pancreatitis. With this diagnosis, the patient was referred back to the clinic, December 29, with the recommendation that an exploratory operation be performed. After repeated examinations, the opinion was that there was insufficient evidence for an operation.

The presence of sugar was generally associated with the severe attacks. The glycosuria was more of a true alimentary glycosuria because its appearance always followed immediately after a heavy meal, and it disappeared in the morning. It ranged from 0.5 to 3 per cent. in amount, quantities far in excess of that found in renal diabetes. Urobilin and urobilinogen were definitely associated with the recurrence of attacks. Bile was found only on two occasions following attacks of unusual severity. When the bile appeared in the urine together with the icteric tinge of the skin, the diagnosis of a lesion in the head of the pancreas was felt to be well established. The enlargement of the gallbladder on several occasions aided in this assumption. Because of the severe colicky attacks, not unlike those of gallstone colic, the most probable diagnosis was pancreatic stone. The slight temperature and increased leukocyte count with several of the attacks suggested an inflammatory condition in the pancreas.

When the exploratory operation was performed, a large tumor mass, mushy in consistency, was found involving the head of the pancreas. Although the mass was not explored at the time of operation, the gross description by the surgeon coincided with the gross description of surgical cases reported in the literature, in which the necropsies showed that the mass was a result of severe, acute inflammation surrounding stones in the pancreatic duct.

Korte [12] reports a case presenting symptoms of biliary colic. The patient was operated on, gallstones were removed and the gallbladder was drained. Shortly after the operation, similar attacks occurred. The patient died. At necropsy, a large tumor was found in the head of the pancreas which at first was thought to be malignant, but on examination proved to be a diffuse abscess surrounding pancreatic stones.

Delageniere [13] reports the case of a man, aged 59, who developed violent epigastric pain followed by increasing jaundice. At operation, the distended gallbladder was opened and a stone removed. The head of the pancreas was large, soft and infiltrated. On incision, calculi were found in the ducts. These were removed, drainage inserted, and the patient recovered.

Although permission for a postmortem was not obtained in the case under discussion, the clinical history as outlined, together with the peculiar mass found at operation in the head of the pancreas, points quite definitely to the diagnosis of pancreatic lithiasis with acute suppurative pancreatitis, the diagnosis originally made when the patient was first seen about six months before his death. In this case also, the principal basis for diagnosis was the peculiar course of glycosuria and blood sugar levels.

Case 3.—Mrs. S. R., aged 64, was referred to me with a diagnosis of severe constipation and diabetes of several years' duration. The present illness started twelve years before with sudden pain in the abdomen and temporary inability to move the bowels; cathartics caused painful evacuations. The first attack lasted for two weeks, and was followed by frequent attacks. The patient now complained of pain in the left side in the lower part of the abdomen, aching and piercing in character, which radiated to the back on the left side and, at times, into the left hip and left knee. She also complained of constipation and loss of appetite.

Physical examination revealed a large mass freely movable in the lower left quadrant. The stools showed an irregular mass, suggesting stenosis along some part of the bowel. Examination of about thirty specimens of urine showed sugar present, from a barely recognizable trace to 17.5 gm. in twenty-four hours, in about 50 per cent. of the specimens. So irregularly did the sugar appear in the urine that it was necessary to resort to special sugar tests to establish the identity of the reducing body. The Cammidge test was performed on a number of specimens, and was found positive in most instances.

A diagnosis of diabetes could not be made because of the inconstancy of the urinary findings on a rather constant diet. Because of the evidence of partial obstruction of the bowels, a laparotomy was performed and a diverticulitis of the colon was found, with an inflammatory mass uniting the sigmoid to the body of the uterus. The patient died the day following the operation. At necropsy, an advanced, chronic interstitial pancreatitis was found, with dense bundles of connective tissue permeating the organ. This case was complicated by two unrelated conditions being present, but the interesting feature was the spasmodic appearance of sugar in the urine, which was not in accord with the ordinary course of diabetes, but for which no true explanation was found. It also is interesting to note that a pancreatic disturbance was suggested by the pathologist examining the specimens of urine.

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12. Korte: Berl. Klinik, December, 1896.

13. Delageniere, H.: De la choledoctomie retropancreatique, pour calculs enclaves dans la portion retropancreatique du choledoque, Arch. prov. de chir. **15**:505-515, 1906.

## CONCLUSIONS

1. Pancreatic disease is more common than is usually supposed.

2. Diseases of the pancreas are diagnosed with difficulty because of its inaccessibility to palpitation, to roentgen-ray studies, to reliable functional tests and to surgical intervention.

3. From postmortem statistics, primary carcinoma is the most common lesion of the gland, with secondary carcinoma, chronic pancreatitis and acute pancreatic necrosis following respectively in order of frequency.

4. Careful studies of the urine for sugar and of the blood for changes in sugar levels and atypical sugar tolerance curves are valuable in diagnosis.

5. Postmortem pathology of the pancreas is generally rather superficial, probably owing to the rapid postmortem changes.

6. Apoplexy of the pancreas is a clinical entity, though rare.

7. Pancreatic atherosclerosis is probably a relatively common condition associated with cases of advanced hypertension and with coronary sclerosis. The diabetes so frequently encountered, in these cases, is probably due almost entirely to this lesion.

8. Diabetes mellitus should always be suspected, but should not be definitely diagnosed from the mere presence of sugar in the urine until appropriate tests are made to establish the diagnosis.

9. No syndrome is pathognomonic of pancreatic disease; one can arrive at the correct diagnosis only through a combination of all the findings.

# Book Reviews

THE THEORY AND PRACTICE OF MEDICAL SOCIAL WORK. By EDNA G. HENRY, Social Service Department, Indiana University. Pp. 195. Ann Arbor, Mich.: Edward Brothers, 1924.

Medical social service has been assuming a position of increasing importance in the last few years until it has become an indispensible adjunct to diagnosis, treatment and teaching. It supplies to the clinician the intimate contact with the daily life and social conditions of the patient which is held by the family physician, and without which, while he may diagnose and treat the disease, he cannot "treat the patient." A clinician in a psychiatric clinic recently stated that diagnosis in the clinic had improved 20 per cent. since the organization of their social service department.

Medical social work must always remain much of an art to be gained by experience; but its methods and aims are scientific, and these may be formulated in a text, as well as the general methods of procedure and the results of the experience of the author.

There has been a very definite need of texts on medical social work, not only for the medical social worker but also for the clinician, hospital and dispensary executives and medical students. This need is met in a large part by the present volume and could be filled by it most successfully if a more detailed consideration were given to certain aspects. This the author is especially well fitted to do.

The purpose and aims of medical social work are well stated, but many of the problems are little more than mentioned, and not enough space is given to suggestions as to procedure. The subject of industrial accidents and diseases is allowed only one-half page, too little to offer much in the way of guidance. Such problems as convalescent care, vocational guidance and training, are properly the functions of special organizations; still they are problems for the solution of which the medical social worker is immediately responsible and should be considered in some detail. In places some suggestions as to procedure are given. This is true of the section on illegitimacy, although the course outlined might be thought more dogmatic than is justifiable. Many social workers have found it very inadvisable for the unmarried mother to keep the child. For the guidance of the student worker both aspects should be considered.

It is hoped that the present volume will have such success that the author and publishers will feel justified in getting out a revised and enlarged edition.

DIABETIC DIET. A HANDBOOK FOR DIABETICS. By H. DORIS MCHENRY, B.A., and MARJORIE M. COOPER, B.A., with a preface by J. A. GILCHRIST, B.A., M.B., and F. G. BANTING, M.C., M.B., M.R.C.S., F.R.C.P., M.D., D.Sc.,LL.D. New York: Harper and Brothers, 1925.

Little "instructor" books for diabetics are almost all following a definite form. The task of teaching diabetic patients how to prepare their own menus is not an easy one; the danger of telling too much is as great as the danger of telling too little. In this book too little is told: the instructions for planning diets seem quite inadequate to reach more than the intelligent patients. Tables of equivalent food values, too, are lacking. The recipes are good.

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